Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

FAO/IAEA
Agriculture & Biotechnology Laboratories

Activities Report **2011**

IAEA Laboratories Seibersdorf
International Atomic Energy Agency
Vienna, Austria

CONTENTS

THE ANIMAL PRODUCTION AND HEALTH LABORATORY

EXECUTIVE SUMMARY

For nearly one billion of the poorest people in the world, livestock is the main asset they rely on for their livelihood. It constitutes not only a source of cash but it also provides manure and ensures traction to support crop production. It also plays inestimably important social and cultural roles for the farmers. So the loss of this asset by diseases, lack of feed and water spells disaster for this vulnerable population. Unfortunately, despite the crucial place that livestock have in effective poverty alleviation policy, only 4% of international aid is directed to agricultural needs in developing countries according to a study of the World Bank. This is an important challenge for international organizations such as the FAO and the IAEA. These organizations, in close collaboration with other organizations and institutions, have succeeded in eliminating the dreadful rinderpest, the first animal disease to be eliminated from the world. One of the factors that has made this success possible is the availability of efficient disease control tools. The main activity of the Animal Production and Health Laboratory (APHL) is to conduct R&D for the development of such tools for the control of animal diseases that are important for the poor: peste des petits ruminants (PPR), the first infectious disease of small ruminants in Africa, the Middle East and Asia. Research was also conducted to develop diagnostic tools for capripox diagnosis, another important disease afflicting ruminants, with the same geographical distribution as PPR. Research that was started in 2010 for the development of a vaccine against trypanosomosis was continued.

In animal genetics, attention was paid to innate genetic resistance to gastrointestinal infections. Indeed, gastrointestinal parasites cause huge economic loss to sheep and goat farmers throughout the world and pose a major management problem for livestock owners. In order to enhance the host resistance against various helminths, APHL pursued, in 2011, its initiatives to develop genotyping tools for association of genetic variations within immune related genes to host response to artificial infection. Similarly, in order to characterize indigenous chicken breeds/populations for their diversity with respect to candidate genes related to immunity, 13 chicken populations were screened for the S631N mutation of the myxovirus resistance gene. Further, APHL, in collaboration with other institutions, developed the radiation hybrid panels for the whole genome mapping of goats. The SNP genotyping of R-H panels in order to construct and publish the first generation whole genome goat radiation hybrid panel map is progressing. In addition, APHL is constantly strengthening the global genetic (DNA) repository of indigenous and commercial livestock breeds, which serves as reference material for collaborative animal genetic research.

In addition to R&D, the second pillar of APHL activities is the contribution to build knowledge capacities in FAO and IAEA Member States. It hosted 3 fellows and conducted two training courses: (i) Major Transboundary and Zoonotic Animal Diseases: Early Detection, Surveillance and Epidemiology, Entebbe, Uganda from 20 June to 1 July 2011; and (ii) Advanced Bioinformatics and Laboratory Data Management for Enhanced Quality Assurance and Quality Control, Vienna, 11–22 July 2011; and a workshop on (iii) Classical and Molecular Veterinary Virology, Vienna from 28 November to 9 December 2011.

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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Animal genetics

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

Gastrointestinal parasitic infestations such as *Haemonchus contortus* and *Trichostrongyle colubriformis* impose severe constraints on sheep and goat production, especially those reared by marginal farmers under a low external input system in the developing world. These parasites cause heavy losses to farmers in terms of body weight loss, direct cost of anthelminthic drugs, loss due to mortality, etc. Emergence of strains resistant to anthelminthic drugs has further complicated the management of parasitic diseases in small ruminants. Breeding programmes with the goal of enhancing host resistance to parasites will help to alleviate these problems in the long term. Hence, the IAEA embarked on promoting a coordinated research project (CRP D3.10.26) on Genetic Variation in the Control of Resistance to Infectious Diseases in Small Ruminants for Improving Animal Productivity. In APHL, initiatives were taken to develop genotyping tools for detecting single nucleotide polymorphisms (SNPs) associated with enhanced resistance to helminths in sheep and goat. An endpoint genotyping system with FRET (fluorescent resonance energy transfer) based KASP (KBiosciences competitive allele specific PCR) system was used to develop SNP typing assays. Initially, 36 novel SNPs were identified across 32 genes involved in immune related pathways and assays were developed to genotype them. SNP typing tests were then validated in a panel of unrelated individuals from different sheep/goat breeds across the world. Further work is being undertaken to identify and

develop genotyping tests for additional SNP markers within other potential candidate genes. These markers will be studied for their association with host resistance in experimentally challenged sheep/goat.

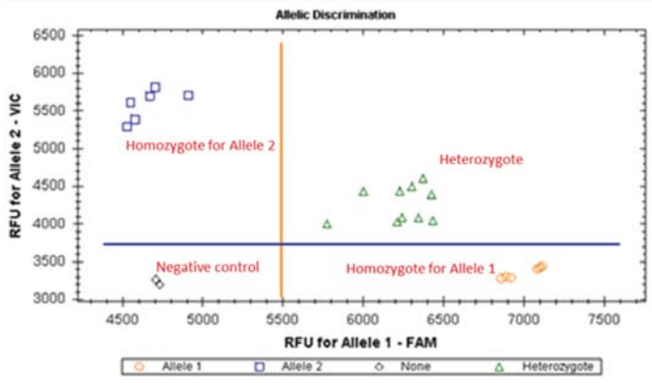

FIG. 1. Endpoint SNP genotyping based on the FRET methodology.

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

Although poultry production has become highly industrialized, use of few breeds/strains in developing commercial chicken has reduced the genetic diversity of chicken worldwide and has contributed to the loss of several valuable traits/characteristics. Currently, the world poultry market is facing recurrent outbreaks of contagious diseases such as avian influenza, *Salmonella, etc.,* which impose serious health and economic constraints. Indigenous chicken populations around the world possess wide genetic diversity and the search for beneficial mutations across important immune related genes in them can be helpful for improving bird resistance to diseases. In this regard, a European regional technical cooperation project was implemented in 2009–2011, which aimed at establishing early bird flu diagnosis and assessment of genetic markers for avian influenza resistance using nuclear and molecular methods**.** One of the objectives of the genetic component of this project was to screen indigenous chicken breeds/strains from different European countries for the distribution of alleles at different candidate gene loci such as myxovirus (Mx) resistance, major histocompatibility complex (MHC), toll like receptors (TLR) and certain microsatellite loci linked to disease resistance in poultry. APHL coordinated the collection of samples from different Member States and developed genotyping assays to screen the genetic variation within indigenous chicken breeds. The Mx resistance gene (Mx/resistance) is one of the important candidate genes with respect to genetic resistance to avian influenza. The presence of the amino acid asparagine (Asn) at position 631 (allele A) has been found to be specific to positive antiviral Mx/resistance, while, that of Ser (allele G) is specific to negative Mx/susceptible. Indigenous chicken breeds, such as Shoumenska from Bulgaria; Hrvatica from Croatia; Green Legged Partridge from Poland; indigenous local chicken from The Former Yugoslav Republic of Macedonia; Aseel and Kadaknath from India; Bangka, Kampang and Gaok from Indonesia and commercial strains such as Lohman White, Lohman Brown, Super Nick White and Ross were utilized for the study. A total of 437 birds distributed across nine indigenous chicken breeds and four commercial chicken strains were screened initially for S631N mutation. The results revealed a high frequency of the supposedly resistant allele (A) in Green Legged Partridge and Kampang

breeds, while the susceptible allele (G) was found to be in high frequency among Kadaknath, Shoumenska, Bangka and Hrvatica chicken breeds. Among commercial chicken strains, Lohman White and Super Nick Layer were found to possess higher frequency of allele A.

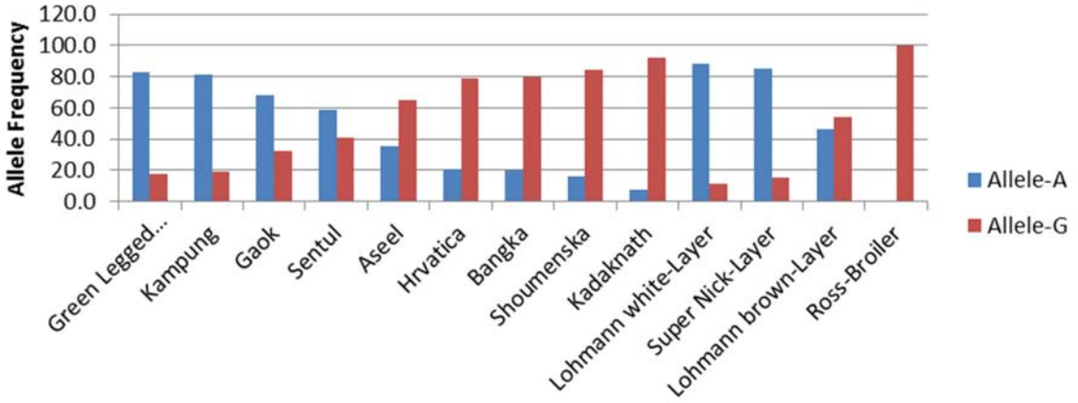

FIG. 2. Distribution of alleles at serine/asparagine mutation locus (S631N) within the Mx gene in different chicken populations.

Whole genome radiation hybrid panel map of goats

Domestic goat is an agriculturally important species and is called poor man's cow because of its vital role in the rural livelihood of many developing countries. Genomic information available on goats is relatively poor compared with other livestock such cattle and sheep. For example, the resolution of the goat gene map available to date is very low, with only few markers being mapped to each of the 30 pairs of chromosomes. The APHL, in collaboration with other institutions, initiated the development of radiation hybrid panels for gene mapping in goats. Radiation hybrid (RH) mapping is a method for producing high resolution maps that can be used for integrating linkage maps and also serves as a link across species for comparative mapping. A 5000 rad goat-hamster whole genome radiation hybrid (WG-RH) panel was generated and preliminarily characterized. A normal diploid fibroblast culture from a male Boer goat was fused with the recipient hamster TK^- cell line, A23. A total of 121 colonies were generated and grown to extract an average of 8.4 mg of DNA per colony. SINE-PCR results showed that almost all colonies retained goat DNA. An optimized panel of 90 RH colonies (CHRH5000) was finally screened as a fundamental tool for advanced goat mapping studies. These clones had an average retention frequency of 34.2%, with 42 markers used in the initial genotyping of the panel. Genotyping of RH panels with SNP chip and construction of WG-RH map is underway. Development of an WG-RH panel will provide a framework map for comparative mapping and for assembly of the goat genome sequence.

Animal health

Development of an ELISA for capripoxvirus

Capripox is an infectious viral disease of ruminants. It is in the list of economically important animal diseases to be notified to the World Organization for Animal health (OIE) in the case of outbreaks. Despite the fact that capripox has a major impact on the ruminant production system in Africa, the Middle East and Asia, where small ruminants constitute a large share

of the agricultural economy, there is no commercially available kit for capripoxvirus specific antibody detection. Such a tool, to be based on the popular enzyme linked immunosorbent assay (ELISA), will assist in the control/eradication programme, screening for international trade and conducting serosurveillance in wildlife. Since 2010, APHL has been addressing this lack of convenient capripox serological assay by putting efforts into the development of a recombinant protein based ELISA. To that end, two proteins were targeted as potential antigens for such a test. In 2011 their genes were cloned and recombinant proteins were produced using an in vitro bacterial system. These recombinant antigens were tested for their potential use in ELISA tests (see Fig. 3).

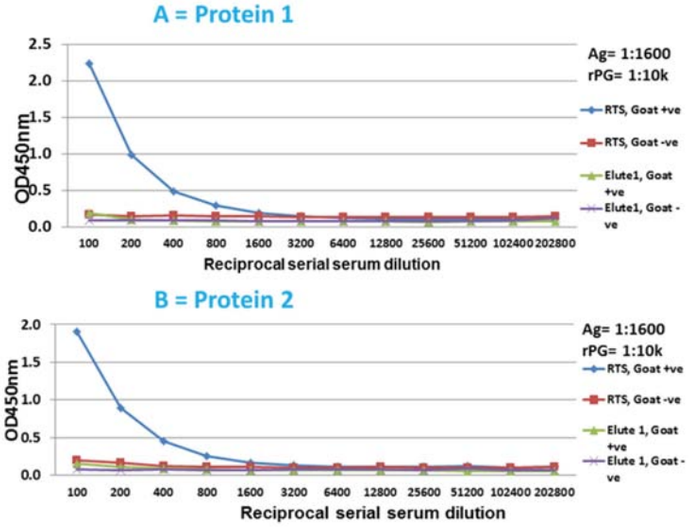

FIG. 3. Performances of two non-purified recombinant proteins for the detection of capripoxvirus specific antibodies.

Each of the two recombinant proteins that were expressed in an in vitro bacterial translation system showed good specificity to capripox infected serum (Fig. 3).

Further work is being undertaken to improve the recombinant protein production and its purification in order to improve the assay sensitivity and specificity and to obtain a production system that will help to fulfil future needs.

Rapid tools for capripoxvirus differentiation

Three viruses, classified in the genus Capripoxvirus within the family *Poxviridae*, are responsible of capripox infection: sheeppox virus, goatpox virus and lumpy skin disease. This classification is based on the animal species origin, since it was believed that these are highly host specific. However, it is now proved that these viruses can cross-infect different ruminant species and cause serious pox disease in sheep, goats and cattle. The APHL has been working for several years now on the development of tools for capripoxvirus differentiation to allow the assignment of the field isolates of capripoxvirus into one of the following 3 groups: sheeppox virus group, goatpox virus group and lumpy skin disease virus group. This is necessary for epidemiological studies and/or the understanding of some vaccination failures recorded in the field: despite the closely antigenic relationship between these viruses, it might

be possible that better protection against a virus is provided when a homologous vaccine is used. For the capripox genotyping without the need of gene sequencing, a gene amplification methodology based on dual hybridisation probes and FRET chemistry was developed in APHL and published in 2011. An alternative method that could be used on any real time PCR platforms was also developed and the paper describing this work is being drafted for publication. FRET chemistry and its alternative are very accurate and allow the simultaneous detection and differentiation of sheeppox virus, goatpox virus and lumpy skin disease virus. However, for all these methods, a pair of labelled probes is needed, adding to the cost. Saturating dye methodologies have now been used for several years for monitoring real time PCR reaction, scanning genes and genotyping microbes. When combined with label free probes for genotyping, similar results to FRET can be achieved. In 2011, APHL undertook the development of a genotyping tool for capripoxvirus using saturating dyes and a label free probe based on Snapback primer technology (Fig. 4).

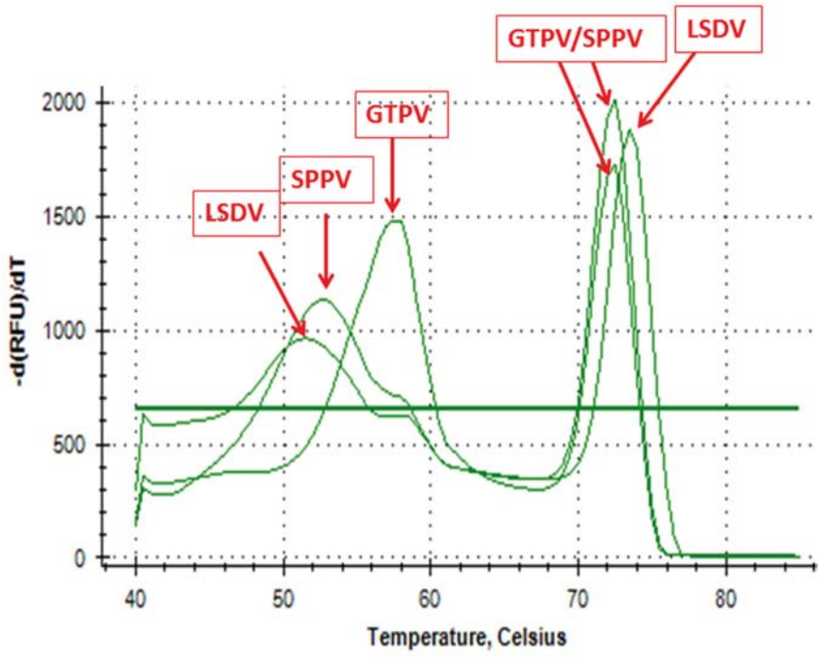

FIG. 4. Fluorescence melting curve analysis of the PCR products. The 3 capripoxvirus species can be differentiated by combining the temperature melting (Tm) information of the PCR amplicons and those of the Snapback probe: GTPV (Tm: 58.00, 72.50), SPPV (Tm: 52.50, 72.50) and LSDV (Tm: 51.50, 73.50).

The initial evaluation of this assay shows that it is very well suited for capripoxvirus genotyping. About 50 samples from various geographical origins were accurately genotyped and assigned into one of the 3 capripoxvirus groups. In addition, the assay doesn't detect non-capripoxvirus DNA; cDNA from PPRV and DNA from parapoxviruses.

This method has good potential for implementation in Member States since it is cost effective and does not need any additional specialized software (such as high resolution melting analysis software) except that of the quantitative PCR (qPCR) machine used to perform the assay.

Molecular epidemiology of capripoxviruses

The understanding of capripox epidemiology and the geographical prevalence of different capripox strains is crucial for the control of the disease. APHL has already developed and published some tools that can assist in molecular characterization of capripoxvirus isolates. The application of these tools in the study of isolates of various origins is giving a more comprehensive picture of the diseases in several regions. They are being systematically used at APHL to investigate new outbreaks as part of its current research work on capripox or on request from Member States. Recently, at the request of a counterpart in Kenya, samples from recent outbreaks in the country were characterized using capripox virus genotyping tools that were developed at APHL. In addition, two genes were amplified, cloned and sequenced for each isolate.

Among the samples that were analysed, 2 samples collected from sheep were characterized as goatpox viruses, adding to the proof that capripoxviruses can cross-infect and cause the disease in different hosts. This study has shown the circulation of two capripoxvirus species during these outbreaks: goatpox virus in both sheep and goats and lumpy skin disease virus in cattle.

Other work undertaken was the molecular survey of various capripox vaccines used in Africa in collaboration with the Pan African Vaccine Centre. The application of both PCR, real time PCR and the sequencing of two genes from each of the ten vaccines that were investigated has shown the predominant use of the cattle strain for the immunization of both sheep and goat, in addition to cattle. Despite the fact that cross-protection exists with capripoxviruses, further studies are needed to understand if this practice should not be reconsidered, especially for some specific capripox which emerge from vaccinated flocks.

Development of specific assay for PPR serological diagnosis

Currently, there are two commercial ELISA based tests available for the detection of antibodies against peste des petits ruminants virus (PPRV). Both are based on the competitive ELISA methodology: the test serum is allowed to compete with a monoclonal antibody (mAb) in binding to an antigen with the competition being quantified by an enzyme linked conjugate which recognizes the mAb. One of the advantages of this methodology, among others, is the possibility to use the same reagents for test serums from different animal species. In general, such a test is highly specific if specific mAb is used in the assay. Unfortunately, there are cases, such as the current two PPR cELISA, where those tests are not highly specific. Indeed, despite the fact mAb anti PPRV is used, both tests show cross-reactivity with sera anti rinderpest virus, a virus closely related to PPRV. As indicated in the introduction of this report, in 2011 rinderpest was officially declared eradicated worldwide. However, the surveillance for rinderpest will continue for many years to come. It is then extremely important to develop an assay that is capable of differentiating between PPR and rinderpest, so that both aspects, namely detecting PPR and surveying for rinderpest, can be achieved in a rapid way. To attain that objective, APHL pursued effort in developing a new cELISA for the specific detection of PPR antibodies. The first step was the production of new monoclonal antibodies against the haemagglutinin protein (H) of the virus. The corresponding gene was cloned and introduced in the bacterial expression vector system for recombinant protein production. This protein was purified and used to immunize two mice. From the spleen cells of one of these mice,

3 hybridoma cells producing monoclonal anti-H PPRV antibodies were generated. One of these cells, clone 12A9.2, was shown to be specific to this protein by using two different techniques: ELISA and Western Blot.

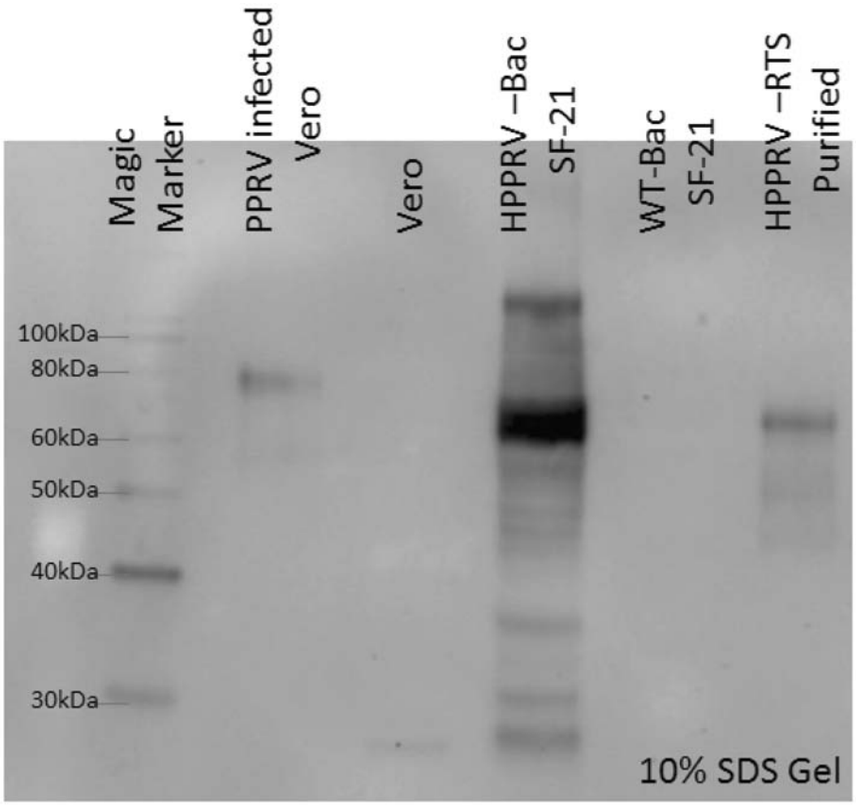

FIG. 5. Western Blot analysis: Characterization of 12A9.2 anti-HPPR monoclonal antibody. 'Bac' and 'RTS' refer to the origin of the host that produces the protein, i.e. insect and bacteria respectively. Vero cells are mammalian cells. H of PPRV produced in mammalian cells is migrating in the electrophoretic gel differently from that of the protein produced in the bacterial or insect system because of the differences in the glycosylation in each system.

Preliminary competitive ELISA (cELISA) studies were carried out in using the mAb anti-HPPRV clone 12A9.2. The results that were obtained indicate that the new mAb competes well with anti-PPRV sera in a specific manner. Further studies are being carried out to determine the ideal conditions for the test. A wide range of samples will also be tested to determine the specificity of the assay.

Molecular epidemiology study of PPRV and validation of the new cell line developed at APHL for PPRV isolation in vitro

Following the development, in 2009, of a cell line highly efficient in PPRV isolation, APHL is receiving PPR suspected pathological specimens from Member States for PPR diagnosis. In 2011, samples were received from Benin, Ghana, Kenya, Pakistan and South Sudan (see Table 1). This diagnostic service was provided by implementing two different types of assay: (i) the reverse transcription-polymerase chain reaction (RT-PCR) to amplify a fragment of a PPRV protein gene, the nucleocapsid protein (N) and (ii) the isolation of the virus in the

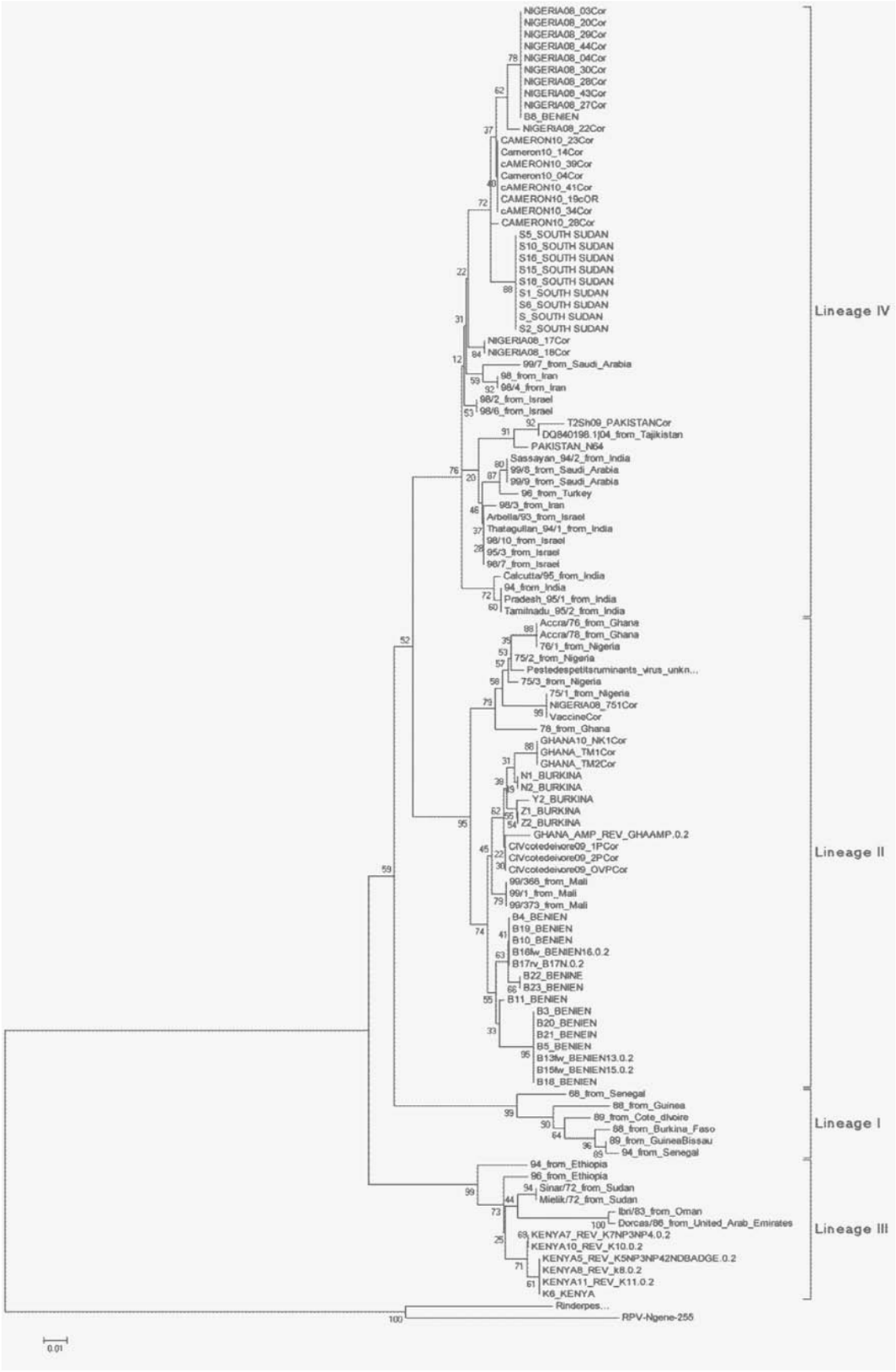

FIG. 6. Molecular epidemiology analysis of PPRV strains based on the partial nucleocapsid protein gene sequence.

cell culture. Table 1 shows the summarized test results. It was an opportunity to continue the validation of the new cell system, named CHS-20. Out of 109 samples that were received, 62 were positive by the nucleic acid amplification assay. From those positive samples, PPRV was recovered by virus isolation from 37. Taking into consideration that PPRV is a fragile virus, it is possible that the live virus was destroyed during the shipment when the specimen was not kept at the requisite cool temperature.

TABLE 1. DETECTION OF PPRV IN PATHOLOGICAL SAMPLES BY RT-PCR AND IN INOCULATED CHS-20 CELL CULTURE BY ISOLATION

Country	Number of received samples	Number of samples			
		Tested by RT/PCR	Positive by RT/PCR	Tested by isolation	Positive by isolation
Benin	23	23	19	19	16
Ghana	35	12	12	17	11
Kenya	20	20	11	7	1
Pakistan	13	11	7	7	7
South Sudan	18	16	13	11	2
Total	109	82	62	61	37

The amplified nucleic acid was sequenced. The data that were obtained were compared with those obtained in 2010 or available from the gene bank. This sequence analysis is summarized in the phylogenetic tree presented in Fig. 6. PPRV is classified into four lineages. Until 2007, lineage I was found only in West Africa, lineage II was in Nigeria and Central Africa, lineage III was in East Africa while lineage IV was the Asian lineage. From Fig. 6, it is clear now that the lineage IV is no longer characteristic of Asia, since it is expanding now in Africa. It is now in North Africa and has reached South Sudan in East Africa and in Cameroon and Nigeria. It has probably not reached Kenya yet, since the virus identified in samples received in 2011 is still of lineage III. While most of the samples from Benin are of lineage II, one is found to be lineage IV, with new Nigeria strains that were involved in outbreaks in 2009. This result has to be confirmed. The PPRV strain identified in the Ghana samples is in lineage II. As expected, the data from the Pakistan samples show lineage IV.

Trypanosomosis

In 2010, APHL started a CRP entitled The Use of Irradiated Vaccines in the Control of Infectious Transboundary Diseases of Livestock, with the aim of developing a non-replicating but metabolically active trypanosome vaccine that is able to induce a protective immune response in the host.

For this study, a recombinant *Trypanosoma evansi* strain expressing the luciferase gene that was obtained from the Prins Leopold Instituut voor Tropische Geneeskunde (Antwerp) is used. The trypanosomes are maintained and propagated in vitro.

The initial studies were done with an equipment emitting Xrays by electrons. The dosage ranges between 50 to 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 550, 600 and 650 Gy.

To measure the viability and metabolic activity of irradiated versus non-irradiated parasites, a combination of microscopy and RNA messenger (mRNA) expression level by in vitro nucleic acid amplification was carried out. For this nucleic acid amplification, a reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was developed in targeting the trypanosome variable surface glycoprotein (VSG).

Figure 7 shows the amplification curves obtained after the irradiation of the parasite at concentrations of 10^4, 10^3, 10^2, 10^1 and 1 trypanosomes/mL. The RT-qPCR was able to detect 1 trypanosome/mL, a level of detection that cannot be achieved by microscopy. Figure 8 shows an example of the decline of parasite total numbers estimated by microscopy at 2, 18, 24 and 48 h post-irradiation at 600 Gy versus mRNA expression. Microscopic counting of parasites 2 h post irradiation did not show any decrease of parasite total numbers (5×10^4/mL, blue curve), but at 18, 24 and 48 hours post-irradiation, no parasites could be counted. Both methods combined proved to complement each other as useful tools in the determination of parasite viability.

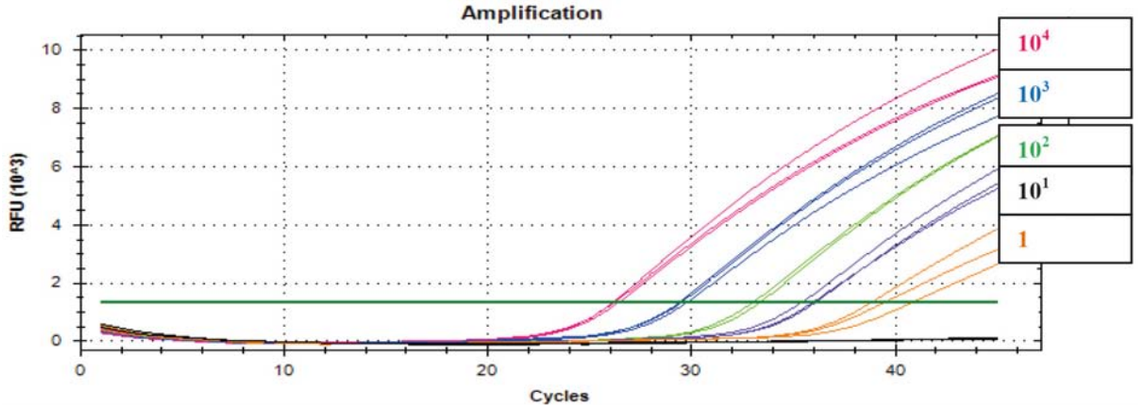

FIG. 7. Quantification of the VSG mRNA after irradiation of trypanosomes at different concentrations: 10^4, 10^3, 10^2, 10^1 and 1 trypanosome/mL (values in triplicates).

The observations made were as follows:

- An irradiation dose of 50 and 100 Gy reduced the total parasite numbers within the first 3 d to almost zero, but some trypanosomes in all wells survived and started to propagate from day 4 onwards. A conclusion on pathogenicity can be taken only after inoculation, e.g. into mice.
- Microscopic examination immediately after irradiation with 200 Gy showed reduced parasite motility and a day-to-day decrease in total parasite numbers. At 72 to 96 h post-irradiation, the parasites in the 55 wells of 24 well plates were dead and some parasites from only one well fully recovered and resulted in normal cell growth and motility. Genomic mutations of the recovered population will be looked for at a later stage.

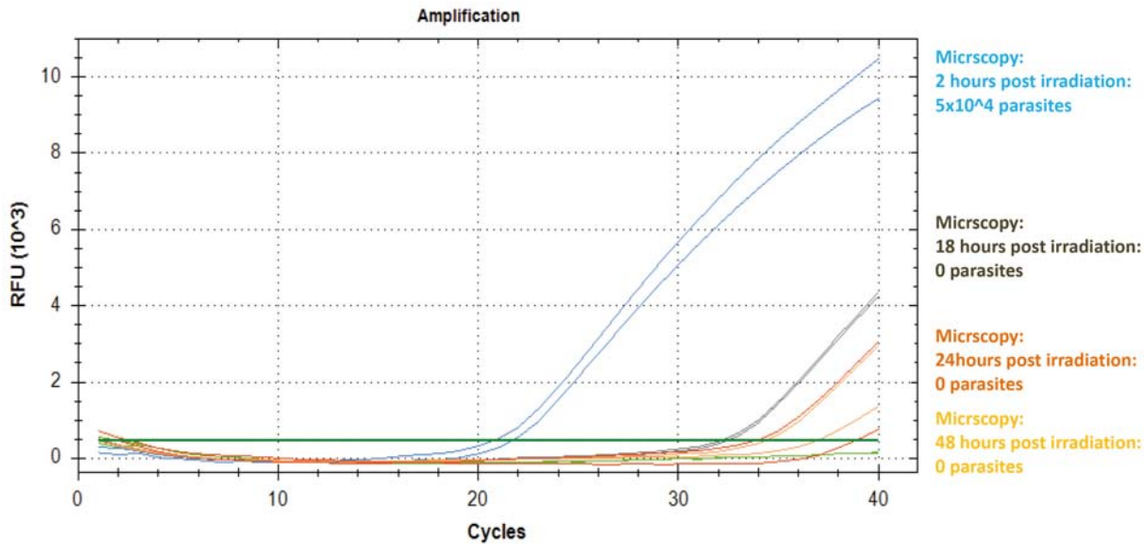

FIG. 8. Quantification of the T. evansi VSG mRNA synthesis after irradiation with 600 Gy in comparison with microscopy results of total parasite number counting.

- Accumulative X ray doses showed different results, than X ray at one shot. The accumulative dosage ranged from 250, 300, 350, 400, 450, 500, 550, 600 up to 650 Gy. The microscopic picture showed shrink parasites with very little motion until week 3 post-irradiation. These deformed parasites did not recover, but RT-qPCR was still positive as an indication of metabolic activity. Actually, this is what we hoped to achieve: non-replicating but metabolically active parasites. These data have to be replicated and verified and tested in mice to investigate the capacity of host immune response induction.

CAPACITY BUILDING

Training courses

A regional training course on Major Transboundary and Zoonotic Animal Diseases: Early Detection, Surveillance and Epidemiology was organized by the APHL in the framework of the US–AID tripartite FAO/OIE/WHO Identify Project at the National Animal Disease Diagnostics and Epidemiology Centre, Ministry of Agriculture, Animal Industry and Fisheries, Entebbe, Uganda, from 20 June to 1 July, 2011. For this 'one health' concept training, one week was dedicated to transboundary animal diseases (CCPP and PPR) and a second to zoonotic diseases (RVF and rabies). Fifteen scientists involved in animal disease diagnosis from Cameroon, Central African Republic, Democratic Republic of Congo, Equatorial Guinea, Ethiopia, Gabon, Kenya, Republic of Congo, Rwanda, Tanzania and Uganda attended this course.

A training course on Advanced Bioinformatics and Laboratory Data Management for Enhanced Quality Assurance and Quality Control was held at the APHL on 11–22 July 2011. The training course consisted of theoretical and practical sessions in the application

of advance bioinformatics tools for viral genome sequence analysis (databases, sequences retrieval, sequences comparison and phylogeny), animal genomic data handling (animal genetic resources databases) and laboratory information management (LIMS, Vet-LIMS). This training was jointly funded by the IAEA TC Project RER/5/015 and the US–AID tripartite FAO/OIE/WHO Identify Project. Nineteen participants from Africa, Asia and Eastern Europe attended this course.

A workshop on Classical and Molecular Veterinary Virology was held in Vienna from 28 November to 9 December 2011. It was jointly organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and the FAO Animal Health Service (FAO-AGAH), the University of Veterinary Medicine (Vienna), the European Society for Veterinary Virology (ESVV), the European Union Epizone Project FP6-2004-Food-3-A, the Network of Excellence for Epizootic Disease Diagnosis and Control (EPIZONE), the European Union project FP6-2005-SSP-5B-INFLUENZA (Capacity Building for the Control of Avian Influenza through Technology Transfer and Training (ConFluTech)), and the European Union funded AniBioThreat project, a project for developing and improving the EU's biopreparedness for risks in the livestock sector and linked to the new EU action plan for hazardous materials and jointly funded by the US–AID tripartite Identify Project and the above mentioned organizers. It aimed at promoting the application of classical virology and multiple pathogen detection methods in veterinary diagnostic laboratories of Africa, Asia and Central and Eastern Europe. The first week, on molecular virology for multiple viral pathogen detection was held at APHL. The second week, on classical virology, took place at the University of Veterinary Medicine in Vienna. Twenty participants from Africa, Asia and Europe attended this course.

Fellowships and scientific visits

Ms Daniela Horvatek from the Faculty of Veterinary Medicine, University of Zagreb, Croatia was attached to the APHL for three weeks (5–23 of September 2011) to work on animal pathogen genotyping techniques.

Mr Kebadire Tlotleng from Botswana was a TC supported fellow (BOT/11001) in the APHL for three months (1 March to 31 May 2011) working on molecular techniques for PPRV diagnostic.

Mr Waqas Ashraf from the National Institute for Biotechnology and Genetic Engineering in Faisalabad, Pakistan, was an intern in the APHL from July 2011 to January 2012. He was trained on the molecular epidemiology of PPR.

PUBLICATIONS

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EXTERNAL COOPERATION AND PARTNERSHIPS

Institution	Topic
Centre de Coopération Internationale pour la Recherche Agronomique et le Développement (Cirad), France	Research on PPR and capripox
Institute for Animal health (IAH), Pirbright Laboratory, United Kingdom	Research on capripox
National Animal Health Diagnostic and Investigation Center (NAHDIC) and the National Veterinary Institute (NVI), Ethiopia	Research on capripox
PANVAC (Ethiopia)	Transfer of animal disease diagnostic test
Laboratoire Central Vétérinaire (LCV), Mali	Research on capripox and PPR
High Security Laboratory, Institute for Veterinary Disease Control, Austrian Agency for Health and Food Security (AGES), Moedling, Austria	Research on exotic animal diseases (capripox and PPR)

FOOD AND ENVIRONMENTAL PROTECTION LABORATORY

EXECUTIVE SUMMARY

The aims of the Food and Environmental Protection Laboratory (FEPL), as a component of the Food and Environmental Protection Subprogramme, are to provide assistance and support to developing countries in their efforts to ensure the safety and quality of food commodities, thereby safeguarding the health of consumers and helping to facilitate international trade. The focus of the FEPL's work is on improving Member State laboratory and regulatory practices and methodologies.

FEPL activities support a holistic food safety approach in Member States. The technical focus is on those aspects of food safety systems for which nuclear and nuclear related techniques can provide a comparative advantage, including issues related to the control of chemical contaminants and residues (pesticides, veterinary drugs, mycotoxins and other natural toxins) in food, and food traceability and authenticity, including the control of food adulteration.

A coordinated research project (CRP) on the implementation of nuclear techniques to improve food traceability commenced in 2011. To support the traceability/authenticity activities, the FEPL continued to investigate the use of robust, affordable and transferrable techniques such as laser spectroscopy to measure $^{18}O/^{16}O$ and $^{2}H/^{1}H$ isotope ratios in water extracted from food products to provide information on their origin. Complementary profiling and marker identification methods using time-of-flight mass spectrometry are also being developed.

Research on the control of food contaminants included the coordination of the CRP on Integrated Analytical Approaches to Assess Indicators of the Effectiveness of Pesticide Management Practices at a Catchment Scale, for which the final RCM was held in 2011. Direct laboratory support to the CRP and to related regional TCPs was provided through the development and transfer of simple, cheap and robust bioassays and biomonitoring protocols to detect contamination of surface water, as well as a number of isotope dilution assays for pesticide residues in food and environmental matrices, and protocols for using ^{14}C labelled pesticides for risk assessment studies. A method was developed for the persistent organic pollutant, dieldrin, in fish samples. The method was applied in a joint research activity with the Insect Pest Control Laboratory related to the control of mosquito populations and is suitable for application in Member States for food safety monitoring.

Work continued in an inter-Agency project aiming to establish a capacity for the quality control of trypanocidal drugs used mainly in sub-Saharan Africa. The use of counterfeit drugs is a cause of significant food security and food safety problems due to ineffective treatment or poisoning of livestock, the development of drug resistance in the disease vectors and the presence of unwanted chemicals in animal derived food products. Methods were elaborated and cross-validated with project partners for the major trypanocidal drugs and monographs incorporating the methodology were prepared for publication through the World Organization for Animal Health (OIE), which will ultimately lead to the adoption of the protocols as international standards.

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. Several simple, cheap, robust, multiple contaminant methods have been developed which will be transferrable under the IAEA Technical Cooperation mechanism.

Presentations on FEPL research were given at three international conferences and the FEPL was involved in the scientific committees of three future major international conferences.

Capacity building activities by the FEPL in 2011 included training to support technology transfer and regulatory policy development under various TCPs and CRPs. More than 190 Member State personnel participated in four train-the-trainers courses and one decision makers/stakeholders workshop implemented by the FEPL in Member States, and three interns and one TC Fellow were trained at the FEPL.

In the immediate wake of the tsunami and consequent nuclear emergency in Fukushima, Japan, in March 2011, the FEPL contributed to the IAEA's emergency response to nuclear and radiological incidents through participation in a fact finding and advisory mission to Japan by a food safety assessment team, led by the FEPL Head, and in follow-up planning for future preparedness and response activities.

Publications by FEPL included nine papers in peer reviewed journals or conference proceedings and four book chapters.

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[a] Left the FEPL in July 2011

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Methodology development for food traceability and authenticity

Awareness of food safety has been rising globally and many importing countries have implemented food control regulations to guarantee the quality and safety of imported foods for their consumers. Food authenticity, adulteration and provenance have evolved to be a major concern in this context. The IAEA has responded with the launch of a CRP on the Implementation of Nuclear Techniques to Improve Food Traceability and the initiation of a regional TCP in south-east Asia focusing on food traceability, both of which include research and development and technology transfer by the FEPL.

Authenticity assessment of fruit juices using ultra-performance liquid chromatography–time-of-flight mass spectrometry

Adulteration of food and beverages is a growing problem in today's global market. Advanced analytical methods are required to protect the rights of producers and consumers with respect to fraudulent practices, including the adulteration of food. These are important issues for legislative, economic and religious or cultural reasons and can also have an impact on food safety. Fruit juices are particularly susceptible to adulteration, as high value juices are often adulterated with relatively low priced juices, which may also have lower nutritional value.

The authenticity of pineapple juice was investigated using ultra-performance liquid chromatography and accurate mass spectrometry. Adulterated samples were prepared in the laboratory by adding 14% of orange juice to pineapple juices.

The experimental data were analysed using principal component analysis (PCA). Obvious separation between the sample groups (pineapple, orange and pineapple-orange mix) was observed, as shown on the scores plot (Fig. 1). This indicated that the samples contained some compounds which are distinctly different and could potentially be used to discriminate between the juices and to indicate adulteration.

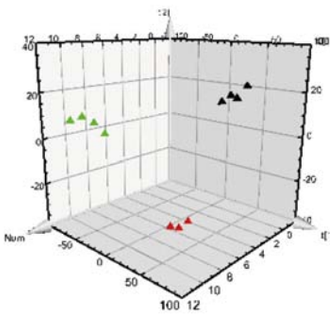

FIG. 1. Grouping of the fruit juices in a PCA 3-D scores plot obtained from pineapple and orange juices (red triangles: pineapple juice adulterated with 14% of orange juice; green triangles: orange juice; black triangles: pineapple juice)

The data were processed using orthogonal projection to latent structures discriminate analysis to identify potential characteristic markers for each juice. Tentative markers with exact retention time pairs were observed from the S-plot (Fig. 2) for pineapple and orange fruit juices. Discriminating marker compounds were identified using database searching. Three compounds (Limonin 17-beta-D-glucopyranoside, narirutin and hesperidin) were identified and selected as markers present in orange juices and one compound (S-sinapylglutahtione) as a marker for pineapple juice.

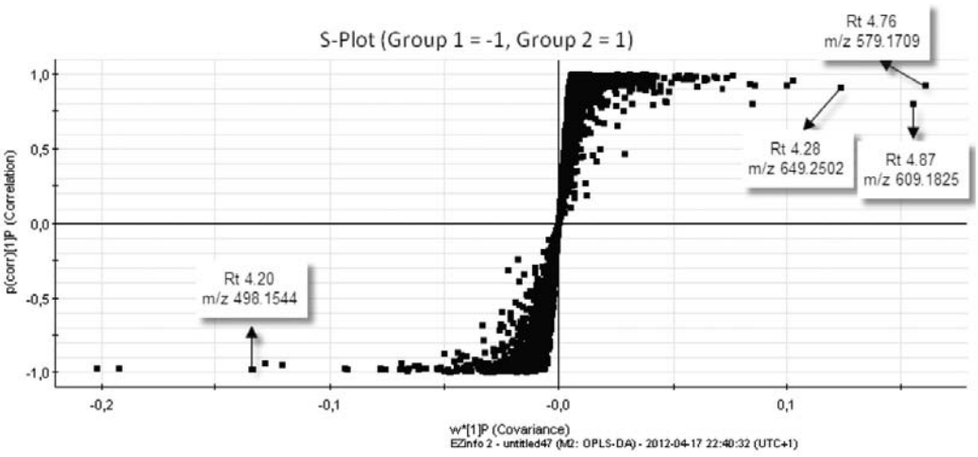

FIG. 2. S-plot of fruit juices to identify characteristic markers (m/z 498.1544, S-sinapylglutahtione; m/z 649.2502, Limonin 17-beta-D-glucopyranoside; m/z 579.1709, narirutin; m/z 609.1825, hesperidin).

Using exact mass measurement and multivariate data analysis, it was possible to discriminate between authentic and adulterated fruit juices and to identify marker chemicals responsible for differences between the groups. The presence of these markers in a fruit juice sample could, therefore, be used to detect the adulteration of pineapple juice with orange juice. The same methodology could be applied to identify discriminatory markers for other juices. Research and development in this field is on-going at the FEPL.

First Research Coordination Meeting for Coordinated Research Project D5.20.37 Implementation of Nuclear Techniques to Improve Food Traceability

The first Research Coordination Meeting (RCM) for the IAEA CRP D5.20.37 was held at the IAEA, Vienna, on 16–20 May 2011.

The main purpose of the CRP is to provide access to skills and know-how and to establish a harmonized system for verification of claims related to food origin, production and authenticity, using nuclear techniques involving mainly isotope ratio analysis and multielement analysis as well as complementary methods.

The meeting was attended by research contract and agreement holders from Austria, Botswana, Chile, China, India, Lebanon, Portugal, Singapore, Thailand, Uganda, United Kingdom and United States of America as well as observers from France, Sweden, the USA, IAEA, FAO and UNIDO.

The meeting included presentations, discussions and drafting sessions. The individual research proposals were reviewed in the context of the CRP's objectives and the strengths and limitations of the individual projects, as well as opportunities for synergistic collaboration, were identified. A common approach and strategy to target the CRP objectives was discussed and agreed upon and individual and overall work plans were prepared.

Key aspects included the establishment of a partner system to enhance contact and interaction between contract holders and agreement holders and establishment of a quality control framework for the CRP. It was recognized that it is of crucial importance to establish validated methods and to provide evidence of the precision and accuracy of the measurements and data produced, including total combined uncertainties, before inclusion of the data in a database. Reference materials must be used where available in order to verify the accuracy of the results. Proficiency testing (PT) is one of the most important tools for proving the 'fitness for purpose' of methods and each laboratory was requested to participate in some form of PT scheme. Method validation and satisfactory participation in a PT scheme are prerequisites for the inclusion of data in the project database.

Another important aspect is the identification and application of appropriate statistical methodologies to interpret the collected data.

It was agreed that the FAO/IAEA eLearning internet platform (http://elearning.iaea.org/ATutor/go.php/143) should be used as an interface for training and as an exchange forum. A planned outcome of the CRP is a database for housing all relevant data for the interpretation of provenance or authenticity of food products, which should be hosted by an impartial institution (preferably the IAEA) and possibly be interlinked with other existing databases.

Sample preparation techniques for laser spectroscopy δD and $\delta^{18}O$ measurements in apples

Work on food traceability using cavity-ring-down spectroscopy (CRDS) continued in the FEPL during 2011. The objective is to develop methodologies for traceability and authentication of food that can be transferred and applied in developing countries. The CRDS instrument installed in the FEPL is a stable isotope analyser based on laser absorption, which measures $^{2}H/^{1}H$ and $^{18}O/^{16}O$ isotope ratios (δD and $\delta^{18}O$) in liquid water. This type of instrument, for certain applications, may offer a robust and affordable alternative to more complex and expensive techniques such as isotope ratio mass spectrometry, especially for field work. The work carried out at the FEPL focused on the optimization of sample preparation methodology to obtain 'pure water' (water free from interfering substances) from fresh fruits and vegetables to allow the accurate measurement of the isotopic ratios by CRDS. Two main approaches were applied: (i) cryodistillation of extracts obtained from homogenized fruits and vegetables to produce a liquid sample and (ii) measurement of the isotopic composition of the vapour phase above the homogenised apple samples in a sealed bag after a defined equilibration time.

The aim of this preliminary study was to assess the correlation between the two sample preparation methods in order to establish a methodology to be used for isotopic traceability and authenticity applications in Member States.

Over a period of one month, apple samples were prepared on seven different occasions according to both methodologies. Statistical evaluation indicated that the results were significantly different on each occasion, the difference between the methods being constant over the replicates. This can indicate that interferences are present and further experiments are needed to optimize the sample preparation for CRDS isotopic analysis. Future work includes investigation of further purification of the sample extract after cryodistillation to remove interfering alcohol traces naturally present in fruit products.

Control of residues and contaminants in food

A number of analytical methods and protocols were elaborated and validated in the FEPL and transferred to Member States through Fellowship training at Seibersdorf, in situ group training or CRP mechanisms.

The analytical methods and laboratory procedures developed are available on the FEP eLearning pages under FAO/IAEA Laboratory Protocols, http://elearning.iaea.org/ATutor/go.php/170.

Integrated monitoring approaches for pesticides in food and water

In response to requests from Member States participating in IAEA Technical Cooperation projects (RLA/5/050 and RLA/5/053), the FEPL contributed to the elaboration of an integrated monitoring strategy to assess agricultural practices and in particular to evaluate the impact of pesticide contamination in food produce and surface waters in selected microcatchment areas. An integrated and multidisciplinary approach is recommended to assess the impact of pesticide management practices in developing countries where pesticide regulations may not exist or lack enforcement. The strategy combines monitoring and modelling approaches using analytical and biomonitoring methodologies to target high impact pesticides in surface water and sediments. Integrated monitoring emphasizes the complementarities achieved by coordinated monitoring using chemical and biological measurements in a variety of environmental media or compartments. Biomonitoring has the advantage of being analogous to watching a movie (temporal process), whereas physical and chemical analyses are more akin to a snapshot in time (instantaneous process).

As part of this work, biomonitoring was identified as an approach to carry out water quality assessments. To this end, the FEPL initiated a study to gain more knowledge about the direct impact of pesticides on aquatic macroinvertebrates, with the goal of distributing a validated, simple rapid-alert tool that would indicate the need for further investigation using complementary physicochemical or nuclear techniques.

FIG. 3. Sampling Gammarus for use in bioindicator experiments in the FEPL.

Samples of shrimp (*Gammarus* species) were collected from an Austrian river (Fig. 3). These were used as local biological indicators as they were abundant and are known from the literature to react to stress created by organic contamination in water. In addition, they are an important food source for many fish species around the world. A protocol was developed for rearing *Gammarus* in aquariums under laboratory conditions. The protocol will be made available to laboratories through publication and in a future training course to be held in Uruguay in November 2012.

Acute toxicity tests using *Gammarus* were performed following the OECD guidelines for testing of chemicals. Representative pesticides were selected based on their widespread use and application rate in Member States (carbofuran, azinphos methyl, chlorpyrifos ethyl) and their

potential to bio-accumulate in organisms (DDT). A range of pesticide concentrations was tested, in the range 0.05–120 µg/L. The LC50 (the concentration that is lethal to 50% of organisms in a given time) values obtained from the experiments were in close agreement with the values published for *Daphnia* in the literature.

An additional experiment was carried out with radiolabelled carbofuran to verify that the mortality of the *Gammarus* in the presence of the contaminant is due to the effect of the pesticide and not caused by radiation from the ^{14}C. This was necessary to enable the planning of bioaccumulation experiments in which feed contaminated with radiolabelled pesticides will be administered to *Gammarus* to estimate the rate of incorporation of the contaminants.

Additional pesticides that are currently used in agriculture in Austria are being evaluated under laboratory conditions for their toxic effects on *Gammarus* species in order to validate the laboratory work with actual measurements using *Gammarus* in situ. This technology makes use of caged indicators which are placed in surface waters for a defined period, for example during the application of agrochemicals in a nearby agricultural field. At the end of the exposure period the cage is recovered and the number of dead indicators is recorded and correlated to the ecological impact of the pesticide applied in the field on the target organism.

Parallel method development in Member States has resulted in the production of a number of bioassay and biomonitoring protocols. For example, a bioassay method using *Daphnia* species for acute toxicity testing is now implemented routinely in Brazil as part of their agricultural practices monitoring. Several complementary physicochemical methods were transferred to Member States; for example, an adaptation of the QuEChERS method for the determination of pesticide residues in pineapple and in soil was transferred to laboratories in Panama and Peru.

A generic guideline for biological monitoring has been prepared and can be found on the FAO/IAEA eLearning web site (http://elearning.iaea.org/ATutor/go.php/135).

Closely related to this work, in 2011, the FEPL concluded a five year international CRP on Integrated Analytical Approaches to Assess Indicators of the Effectiveness of Pesticide Management Practices at a Catchment Scale. The CRP brought together analytical laboratories who are members of wider and multidisciplinary groups. This network provided the assessment of good agricultural practice at a catchment scale using harmonized and integrated approaches to monitor the presence of selected high-impact-ranking pesticides in surface water and sediments. The analytical capabilities of these laboratories, enhanced through the CRP and technology transfer of more than 20 analytical methods from the FEPL, have now been recognised by the regional stakeholders resulting in requests for technical advice and dialogue on policy issues. The established analytical laboratory network facilitated inter laboratory proficiency testing in most participating countries. Moreover, the network now provides a platform for the continuous identification of areas of improvement necessary for enhancing food and environmental safety, especially in the Latin American and Caribbean regions. Areas for improvement that have been identified through this project by the analytical laboratories are now being shared with decision makers responsible for risk management. For

example, feedback to stakeholders on estimates of environmental risk due to pesticide use has resulted in changes in management practices with consequent added value in the food production chain.

Development and validation of a GC-ECD method for the determination of dieldrin in fish samples

Dieldrin is an insecticide that was widely used throughout the world from the 1950s to the early 1970s for cotton and maize farming and other pest control purposes (e.g. locust and mosquito control). Dieldrin is a persistent organic pollutant, highly resistant to biodegradation and abiotic degradation, and is known to biomagnify along the food chain. Long term exposure is toxic to a wide range of animals, including humans, producing carcinogenic and teratogenic effects. For this reason dieldrin is now banned for agricultural use in most of the world, but still has some restricted applications in various countries.

In agricultural areas, cultivated fields can be located very close to water streams, increasing the possibility of pesticide contamination of the environment on a large scale. Incorrect use of pesticides can also contribute to their dissemination. Dieldrin may also occur in the environment through the oxidation, under normal environmental conditions, of aldrin, which is also an insecticide. Dieldrin adsorbs onto soil and sediments and may be distributed in water sources and accumulate in biota.

One possible contemporary use of dieldrin is in a protocol for the control of mosquito populations through the application of the sterile insect technique. In this context, the FEPL collaborated with the Insect Pest Control Laboratory in a study to investigate the potential contamination of fish feeding on mosquitos treated with dieldrin and its bioaccumulation in the fish tissue. A simple and inexpensive method was developed in the FEPL, using gas chromatography coupled with an electron capture detector (GC-ECD), a technique readily available in many Member States, to identify and quantify residues of dieldrin at low contamination levels in different fish species. The isolation of the compound of interest from the fatty fish matrix is problematic because fish lipids and proteins are typically co-extracted, causing significant matrix effects that make identification and quantification difficult and contaminating the analytical column and detector. Appropriate sample/extract cleanup steps are essential.

The extraction method developed in FEPL employs a very simple and fast procedure in which the fish samples were homogenized before being diluted with water and extracted using dispersive solid phase extraction with acetonitrile and a mixture of clean-up salts. The method is linear over the concentration range 2–20 ng/g, accurate, with recoveries ranging between 87.5 and 105.8%, precise (CV < 6.6%) and sensitive, with a limit of quantitation of 2 ng/g (Fig. 4). The method is suitable for application in Member State laboratories. Investigation of the distribution of dieldrin in different fish species from streams and rivers, especially in developing countries where dieldrin may still be available for restricted use, would provide valuable information on the presence of this contaminant in the aquatic ecosystem and in freshwater fish used as foods and on the possible risks for local population.

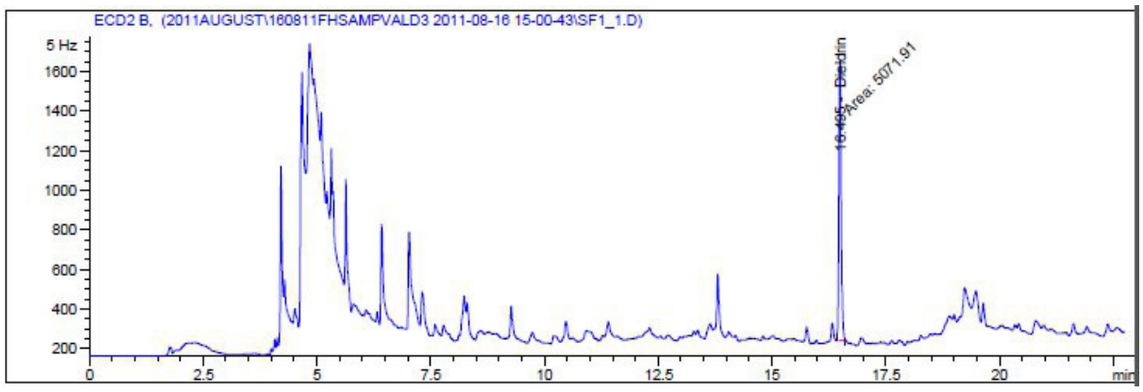

FIG. 4. GC–ECD chromatogram showing dieldrin detected in a fish tissue extract.

Combatting the black market in counterfeit veterinary drugs

The FEPL continued to collaborate in a project led by the International Federation for Animal Health (IFAH) and the FAO, which are working closely with the OIE to support the setting of internationally accepted pharmaceutical standards or monographs for veterinary medicines used in the prevention and treatment of animal African trypanosomosis, a fatal disease that has devastating effects on animal and human health, economic development and food security in sub-Saharan Africa. Through the Programme Against African Trypanosomosis, key African institutions, including the African Union's Inter-African Bureau for Animal Resources and Pan African Tsetse and Trypanosomiasis Eradication Campaign, have been involved in this initiative.

According to FAO estimates, animal African trypanosomosis, more commonly known as Nagana, causes three million cattle deaths every year. The economic loss in cattle production is estimated at a staggering US $4.5 billion annually, which is further compounded by decline in crop production due to the impact on work animals necessary for cultivation. Although medicines are available to prevent and treat the disease, safeguarding animal health is threatened by the sale of sub-standard and counterfeit, and potentially detrimental products. According to IFAH estimates, the black market trade amounts to approximately US $400 million every year.

IFAH and the FAO joined forces to lead an international alliance to develop internationally and scientifically agreed pharmaceutical monographs for trypanocidal products, together with protocols for their quality control and quality assurance. These monographs and the analytical methods to support them have been elaborated and validated by the FEPL in collaboration with the Institute of Pharmacy and Biomedical Sciences of Strathclyde University, UK. These monographs and analytical protocols will be made publicly available as an international reference in an annex to an article in the OIE's peer reviewed Scientific and Technical Review series in 2012, while awaiting the OIE procedure on including monographs of veterinary products into the standard setting process.

Interaction with the EU 7th Framework Research Programme

The FEPL continued to interact with the EU's 7th Framework Research Programme in 2011 in an advisory capacity for the integrated project Contaminants in Food and Feed: Inexpensive Detection for Control of Exposure (CONffIDENCE), for which the Laboratory Head chairs the Advisory Board. The third annual meeting of the CONffIDENCE consortium was held in Belfast, UK, in March 2011.

CONffIDENCE is a 4-year project with 17 partners from 10 countries and a budget of €7.5 million, of which €5.8 million is from the EC. The main objective of the project is the development of novel, multiplex screening methods for a wide range of contaminants in high risk products such as fish and cereal based food and feed, and vegetables. The validated methods will be applied to provide data for risk assessment and for regulatory systems for food safety. The project has resulted in the development of several simple, cheap, robust and portable test methods to enhance food safety both within and outside Europe. The methods will be validated in the final phase of the project in 2012. Some of these test methods will be transferrable to Member States through the IAEA's Technical Cooperation mechanism.

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

Dissemination of research results

The results of research performed at the FEPL, or in collaboration with partner laboratories, were presented at several international conferences and seminars in 2011, including:

- A keynote lecture on Food Integrity and Traceability — the Developing Country Perspective and two posters on research performed in the FEPL: (i) Simultaneous determination of tropane alkaloids and glycoalkaloids in seeds and grains by liquid chromatography-tandem mass spectrometry and (ii) A cost-effective scheme for developing countries to improve food safety using environmental indicators of good agricultural practices at a catchment scale, were presented at the international conference Food Integrity and Traceability, Belfast, UK, 21–24 March 2011. The event brought together more than 240 scientists, policymakers and industry representatives from 25 countries, representing all five continents, to share research, knowledge and expertise and discuss the key topics relating to the challenges and progress in maintaining and improving the safety and integrity of the food supply.

- An oral presentation entitled "FAO/IAEA initiatives for food contaminant control in developing countries" and a poster on "Uptake of ^{14}C-atropine from soil by wheat and its translocation to shoots" at the 2nd Saskatoon International Workshop on Validation and Regulatory Analysis (SaskVal II), Saskatoon, Canada, 19–22 June 2011.

- Two posters: "A coordinated research project on the implementation of nuclear techniques to improve food traceability" and "Uptake of ^{14}C-atropine from soil by wheat and its translocation to shoots" at the 5th International Symposium on Recent Advances in Food Analysis (RAFA), Prague, Czech Republic, 1–4 November 2011. This is the leading international conference in this field. The 5th symposium focused on recent advances in analytical and bioanalytical technologies and emerging food related applications.

In 2011, FEPL was also involved in planning the scientific programme of international conferences through the Laboratory Head's inclusion in the scientific committees of:

- The International Conference on Food Integrity and Traceability, Queen's University Belfast, UK, 21–24 March 2011;

- The Saskatoon International Workshop on Validation and Regulatory Analysis, Saskatoon, Canada, 19–22 June 2011;

- The Euroresidue VII Conference on Residues of Veterinary Drugs in Food, Egmond aan Zee, Netherlands, 14–16 May 2012.

CAPACITY BUILDING

The FEPL provided technical management for 11 national and 3 regional technical cooperation projects (TCP) in 2011. The expertise available in the 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. More than 190 Member State personnel participated in four training courses and one decision makers/stakeholders workshop implemented by the FEPL in Member States in 2011, and three interns and one TC Fellow were trained in the FEPL. In addition, a staff member from the FEPL carried out a mission to Oman to advise on the development of a control system for pesticide residues in food.

Training courses implemented by the FEPL included on-site training for the staff of the Belize Agricultural Health Authority's Central Investigation Laboratory on pesticide residue analysis in fruits, vegetables, soil and fish products, including the use of radiotracer techniques. Regional training courses for the Latin America/Caribbean region were held in Valdavia, Chile, on Bioassays and Bioindicators for the Control of Pesticides and other Chemical Contaminants in Food and the Environment, funded by TCP RLA/5/053, and in Lima, Peru, on QuEChERS Sample Preparation and Liquid Chromatography-Mass Spectrometry Analysis for Pesticides in Food and the Environment. A training course on mass spectrometric techniques for the control of pesticide residues in food was held in Hangzhou, China.

A decision makers and stakeholder regional workshop was held in San Jose, Costa Rica, in conjunction with an RLA/5/053 project meeting. The workshop addressed the central role played by analytical laboratories in the implementation of good agricultural practices and the

control of contaminants in food and the environment and strategies to optimise the impact of technology transfer and policy advice provided through projects run by the IAEA and other organisations. The meeting had approximately 80 participants, including delegates at ministerial and ambassadorial levels, as well as policy makers at national level, regulators and NGO representatives.

The FEPL hosted three interns in 2011. Ms Malia Galluccio and Mr Wolfgang Dieter Werner, from EARTH University, Costa Rica, commenced internships in September. Malia and Wolfgang are working with FEPL staff on the development of bioassays and biomonitoring 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 will be transferred to Member States through a number of regional and national TCPs. Mr Sorivan Chhem-Kiethand, from Concordia University, Canada, extended his internship by six months to work jointly with the FEPL and the 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.

Two TC Fellows were trained during 2011. Ms Moe Thida Kyaw, from the Department of Medical Research in Myanmar, officially hosted by the Plant Breeding and Genetics Laboratory, was trained in the FEPL on analytical QA/QC for approximately two weeks. This included hands-on experience in sample preparation and analysis using high performance liquid chromatography with diode array detection, as well as troubleshooting. She will use this experience in Myanmar for nutrient analysis. Mr Khaled El-Hawari commenced a 4-month TC fellowship in the FEPL in October 2011. Khaled worked on the development of methods for the traceability of food and feeds using stable isotope ratio analysis, primarily focusing on the analysis of water extracted from agricultural commodities and analysed for δD and $\delta^{18}O$ using cavity ring-down laser spectroscopy. Khaled is a previous trainee in the FEPL in the field of pesticide residue analysis, and his return to train and contribute to the FEPL's work on stable isotope analysis reflects the widening scope and success of his home institute, the Laboratory for the Analysis of Pesticides and Organic Pollutants of the National Council for Scientific Research, Lebanese Atomic Energy Commission, with assistance from the FEPL under the IAEA's Technical Cooperation programme.

Emergency preparedness and response

The magnitude 9 Tohoku earthquake off the east coast of Japan and the subsequent tsunami on 11 March 2011 resulted in damage to the nuclear power plant at Fukushima Dai-ichi with the consequent release of radioactive material into the environment. Air, soil, water and agricultural produce around the damaged plant were contaminated with radionuclides, chiefly ^{131}I, ^{137}Cs and ^{134}Cs.

The Japanese Government accepted early offers of assistance from the IAEA in the form of radioactivity monitoring teams to complement and verify the data produced by the Japanese authorities. On 26 March, a joint IAEA/FAO food safety assessment team (FSAT) was also fielded. The team was led by the Head of the FEPL and included a soil scientist from the Joint FAO/IAEA Division's Soil and Water Management and Crop Nutrition Laboratory

at Seibersdorf and a senior officer of the EMPRES Food Safety, Nutrition and Consumer Protection Division, FAO.

The mission provided advice and assistance to the Japanese authorities on technical issues related to food safety and agricultural countermeasures, including sampling and analytical strategies and interpretation of monitoring data to ensure that reliable, continuous updates could be provided on the extent of food contamination in affected areas. These data would form the basis for the development of mitigation and remediation strategies.

The FSAT met with local government officials and stakeholders in the agriculture sector in the four prefectures most affected by the nuclear emergency: Fukushima, Ibaraki, Tochigi and Gunma. The team provided relevant technical information and advice in question and answer and discussion sessions, including information on possible mitigation and remediation techniques and strategies that could be employed on a case by case basis depending on the level of contamination, soil type, land use and other relevant factors. The team also observed agricultural production practices and land in the vicinity of the four prefecture capitals and visited a typical farm producing spinach and rice in Tochigi Prefecture.

The lessons learned from the nuclear emergency in Japan are being used to improve the capabilities of the IAEA and other United Nations organizations to respond effectively to possible future occurrences of this type of situation. The incident provides an opportunity for research programmes to be instigated to collect information in the immediate and mid- to long term that could be used to characterise agricultural contamination and to improve the effectiveness of agricultural countermeasures.

PUBLICATIONS

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EXTERNAL COLLABORATIONS AND PARTNERSHIPS

Institution	Topic
Laboratorios Microbioticos s/c/ Ltda, Sao Paulo, Brazil	Method development for food contaminants, technology transfer to Latin America
University of Costa Rica (UCR), Centro de Contaminacion Ambiental (CICA), Costa Rica	IAEA Collaborating Centre for eLearning and accelerated capacity building for food and environmental protection (EACB)
Institut für Lebensmittel Arzneimittel- und Umwelt-Analytik (ILAU), Germany	Collaborations on research activities linked to the CRP D5.20.35 on Integrated Analytical Approaches to Assess Indicators of the Effectiveness of Pesticide Management Practices at a Catchment Scale
Commonwealth Scientific and Industrial Research Organisation (CSIRO), Division of Land and Water, Groundwater Management and Site Remediation, Australia	
Ministry of Health, State General Laboratory, Environmental Chemistry, Ecotoxicology, Pesticides and Radioactivity Department, Cyprus	
Austrian Agency for Health and Food Safety (AGES), Austria	Collaboration on accelerated capacity building for risk analysis and contaminants in food
Gartner & LVA Analytik, Austria	
Austrian Institute of Technology, Seibersdorf, 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, United Kingdom	Research and method development activities for food contaminants and food traceability
ASSET Centre, Queen's University, Belfast, United Kingdom	Research activities in isotope ratio methods for food traceability
International Union of Pure and Applied Chemistry (IUPAC), Chemistry and the Environment Division	Collaboration on compendium of agrochemicals information
Waters Corporation, Milford, MA, USA	Training for Member State scientists in analytical techniques

Institution	Topic
Agilent Technologies, USA	Training of Member State scientists in analytical techniques
RIKILT Institute for Food Safety, Netherlands	Research into causes of food contamination with veterinary drug residues
Chinese Academy of Agricultural Sciences (CAAS), Institute for Application of Atomic Energy, Department of Agro-Ecological Environment, China	Development of methodology for food traceability and residue 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
International Federation for Animal Health	Quality control of trypanocidal drugs in sub-Saharan Africa
UNIDO	
UNODC	
University of Strathclyde, Glasgow, United Kingdom	

INSECT PEST CONTROL LABORATORY

EXECUTIVE SUMMARY

The work of the Insect Pest Control Laboratory (IPCL) revolves mainly, but not exclusively, around three major pillars: research and development, capacity building and services.

The research and development component currently focusses on work with three insect groups, tsetse flies, fruit flies and mosquitoes. Research with tsetse flies continued in support of the *Glossina palpalis gambiensis* tsetse eradication project in the Niayes of Senegal. To prepare for aerial releases of sterile male flies, field cage studies were carried out to assess the impact of pupal chilling, irradiation and adult fly chilling (to simulate the chilled conditions in the aerial release machine) on the competitiveness of the male flies. The data indicated that the treated male flies were less successful in securing a mating with females than untreated males.

Work continued on the *Glossina pallidipes* salivary gland virus that causes salivary gland hypertrophy (SGH) and reduces fly productivity. A clean feeding strategy (i.e. feeding all flies on fresh, clean and unused blood using a clean membrane and blood tray) used for one year significantly reduced horizontal transmission and the prevalence of SGH in the colony. Adding the antiviral drug, valacyclovir, to the blood meal for 80 weeks significantly reduced the prevalence of SGH and the viral load in the flies. In field cages, SGH negatively impacted the sexual performance of male *G. pallidipes* with males with SGH being less successful in mating when competing with males without SGH. Females mated with males with SGH did, however, not show an increased tendency to remate.

A study assessing the prevalence and genetic diversity of the virus in various *G. pallidipes* populations in East Africa revealed that genetic diversity of the virus was low with random distribution among the virus haplotypes. It is, therefore, feasible to develop and apply common antiviral strategies in different laboratory colonies of *G. pallidipes* regardless of the virus haplotype in circulation.

Research was initiated to unravel the association between the complex of symbiotic bacteria and the prevalence of the virus. Initial results suggest that decimating the bacterial symbionts and, hence, suppressing the innate defence of the fly plays either no role or a minor role in regulating replication of the virus.

In the fruit fly group, the mating compatibility between populations belonging to the *Anastrepha fraterculus* complex from Jalapa (Mexico), Piura (Peru), Vacaria, Piracicaba and Pelotas (Brazil) and Tucumán (Argentina) was assessed. Sexual isolation was observed in the Piura–Pelotas, Piura–Vacaria, and Piracicaba–Tucuman combinations, whereas a tendency to random mating was observed in the Tucuman–Jalapa, Pelotas–Jalapa and Piracicaba–Jalapa combinations.

The IPCL is collaborating with the United States Department of Agriculture on the development of phytosanitary and regulatory treatments for exotic tephritid fruit flies. Third

instar larvae of the peach fruit fly *Bactrocera zonata* (n = 36 820) that had infested navel oranges and that were treated for 18 d at temperatures not exceeding 1.7°C did not survive the treatment. These experiments provide strong evidence that an 18 d cold storage treatment of oranges or other citrus varieties at temperatures not exceeding 1.7°C will safely mitigate the risk of introducing *B. zonata* into the USA.

Radiation studies were pursued with *Aedes albopictus*, an important vector of dengue and chikungunya. A radiation dose of 40 Gy completely sterilised male *Ae. albopictus* but competitiveness of the males was low (0.15) when males were young (1 d). Competitiveness increased (0.59–0.82) when males were kept in the laboratory for 5 d before being released. Four day old sterile male *Ae. albopictus* were as successful as un-irradiated males in inseminating females, but when males were given 10 females every day for 15 d, the insemination success of irradiated sterile males was lower as compared to those that were un-irradiated.

A new larval mosquito diet consisting of a mixture of tuna meal, bovine liver powder, squid liver powder, brewer's yeast and a small addition of vitamin mix was tested for use with *Ae. albopictus*. Larval periods were shorter and a significantly larger proportion of the total males appeared within the first 24 h of pupation than those fed a diet that consisted of cat food, brewer's yeast and fish food.

A new method was developed to digitally quantify large numbers of eggs (>10 000).

Work was also pursued with *Anopheles arabiensis*, which is an important vector of malaria. Radiation and dieldrin treatments (to eliminate the female mosquitoes) reduced the initial sperm stock, and sperm production decreased with male age in the irradiated group. Sperm production in dieldrin-treated/irradiated males was higher at day 6 as compared to males who were only irradiated, suggesting that the dieldrin treatment might have a radiation protectant effect.

In some countries, mosquito pupae will be irradiated at the production site before being transported to the field site for release. To prevent emergence during transport, the development of the pupae could be delayed by storing the pupae at low temperatures. Storing *An. arabiensis* pupae for 6 h at 12˚C sufficiently delayed adult emergence without negatively affecting viability or reproductive ability.

The effect of dieldrin treatments of larvae of the genetic sexing strain of *An. arabiensis* (IPCL1) on adult male longevity was assessed. The analysis showed that the treatment with dieldrin did not affect the longevity of the IPCL1 males.

With respect to capacity building, in 2011, the IPCL hosted ten cost-free experts, six consultants, three interns, two PhD students and six fellows, the latter funded by the Department of Technical Cooperation.

In 2011, the IPCL supplied tsetse fly pupae to six research institutes in Slovakia, Germany, Switzerland, the United Kingdom, Italy and the USA, and fruit fly eggs and pupae to 13 research institutes in France, Portugal, Brazil, Greece, Slovakia, Italy, Malaysia, Chile, Turkey and the USA.

STAFF

Name	Title
Vreysen, Marc	Laboratory Head
Abd Alla, Adly	Virologist (tsetse flies)
Franz, Gerald	Geneticist (plant pests)
Gilles, Jeremie	Entomologist (mosquitoes)
Vacant	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
Kurtulmaz, Elsa	Data Entry Clerk
Wimberger, Tamara	Team Assistant

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Tsetse flies

Mating competitiveness of chilled Glossina palpalis gambiensis

Upon request of the Government of Senegal, the Insect Pest Control laboratory (IPCL) carried out research in support of the *Glossina palpalis gambiensis* tsetse eradication project in the Niayes. A handling and transport protocol to transport chilled male pupae from Bobo Dioulasso, Burkina Faso to Dakar, Senegal that was based upon research carried out at the IPCL was developed and validated in the field. The research assessed the impact of chilling male pupae at various temperatures and various periods on their productivity and competitiveness. Ground trial releases carried out in 2011 with male flies that were transported using the above mentioned protocol indicated adequate competitiveness of the sterile male *G. p. gambiensis* in the habitat of the Niayes (Baba Sall, Momar Seck, Jeremy Bouyer, personal communication).

The project in Senegal is now preparing to enter the operational phase that will include mass releases of sterile males from the air. A new aerial release machine has been developed that will allow the release of adult males in chilled conditions at low release densities. Research was carried out at the IPCL to assess the impact of the additional chilling of adult flies on their performance.

To simulate the entire handling procedure, male *G. p. gambiensis* pupae from the Burkina Faso strain were chilled at 10°C for 5 d and irradiated with 110 Gy just prior to emergence and the adult males again chilled 6 d after emergence for 6 or 30 h. The competitiveness of the male flies was tested in field cages set up in an environmentally controlled insect greenhouse (24°C and 60% relative humidity (RH) and natural light intensity of 300–5000 Lux). The chilled and irradiated males competed with 6 d old males that had emerged from pupae that were incubated at 24°C and 75% RH for mating opportunities with females.

Significantly more untreated males were successful in mating than chilled males. The premating time and duration of copulation were not significantly different when males were chilled at 10°C for 6 h. When the males were chilled for 30 h premating time was significantly longer compared to the control.

Mating performance of male Glossina palpalis gambiensis *in relation to time of day*

A trial to compare morning and afternoon mating performance of *G. p. gambiensis* in a controlled environment was conducted. The field cage was set up under fluorescent lights in a room with ceramic tiles on the walls and floor, with regulated temperature (24–25°C), relative humidity (60–65%) and light intensity (550 Lux). Observations were carried out from 0900 h to 1200 h and from 1400 h to 1700 h.

Mating parameters checked were premating time, mating duration and spermathecal fill. There were similarities in all of the parameters irrespective of time of day.

Salivary gland hypertrophy virus in Glossina pallidipes

Some tsetse species carry a virus that, in a certain proportion of individuals, leads to salivary gland hypertrophy (SGH) and these individuals show reproductive abnormalities. In natural populations, the prevalence of the virus is low (0.5–5%), based on salivary gland dissection. In a colony of *G. pallidipes* that originated from Uganda and is maintained at the IPCL, the frequency of SGH ranged from 4 to 10%. However, polymerase chain reaction (PCR) analysis confirmed that the virus is widely distributed in laboratory colony flies, e.g. the virus caused a very high prevalence of SGH (>70%) in a *G. pallidipes* colony that originated from Arba Minch and was maintained at the Kaliti facility in Ethiopia. The high SGH prevalence was associated with poor performance of the colony.

Due to the negative impact of the virus on colony productivity, it is important to better understand the virus and its interactions with the host to be able to develop appropriate strategies for its management. As a first step, the genome of the virus was sequenced and the information obtained provided opportunities for: (i) the use of PCR and quantitative PCR (qPCR) to analyse the prevalence and dynamics of the virus; (ii) the production of virus specific antibodies that could be used to neutralize the virus infection; and (iii) designing and generating oligo-peptides that could impede the virus infection. In addition, work was carried out on: (i) the effect of selected antiviral drugs to suppress viral replication; (ii) virus transmission in the colony; and (iii) the impact of clean feeding on virus prevalence. Finally, the virus prevalence in wild tsetse populations collected from different African countries was assessed by PCR and the genetic variability among the virus isolates was analysed based on the sequence of six conserved genes.

Dynamics of the virus in a Glossina pallidipes colony in relation to clean and contaminated feeding

The high prevalence of the SGH virus (SGHV) in *G. pallidipes* colonies maintained at the IPCL and in the mass rearing facility in Kaliti, Ethiopia in comparison to the relatively low prevalence under field conditions has been the subject of many questions related to virus transmission. It was concluded that vertical transmission, from mother to progeny, is the main mode of transmission in nature, whereas horizontal transmission, from one fly to another, occurs mainly in laboratory colonies where the in vitro membrane feeding system is used. Several experiments have confirmed the transmission of the virus via contaminated blood to infect other flies and this knowledge was used to develop virus management strategies, i.e. to neutralize the virus infection using virus specific antibodies and using ‘clean’ blood feeding, to block horizontal transmission.

To assess the validity of this concept, two clean feeding colonies were established: (i) a clean feeding colony 1 — all flies were fed on fresh, clean, unused blood using a clean membrane and tray for each blood meal; and (ii) a clean feeding colony 2 — all flies were fed on the blood used for feeding clean feeding colony 1, and the remainder of the colony was fed on the blood previously used by the clean feeding colonies (denoted ‘contaminated feeding’).

After one year, a significant decrease in the prevalence of SGH in clean feeding colony 1 was observed compared to the contaminated feeding colony and a significant increase in SGH in

the contaminated feeding colony (up to 20%) compared to the average prevalence in the colony recorded over several years (5%) (Fig. 1). These results are indicative of the close relationship between the feeding system and the dynamics of virus infection in a *G. pallidipes* colony.

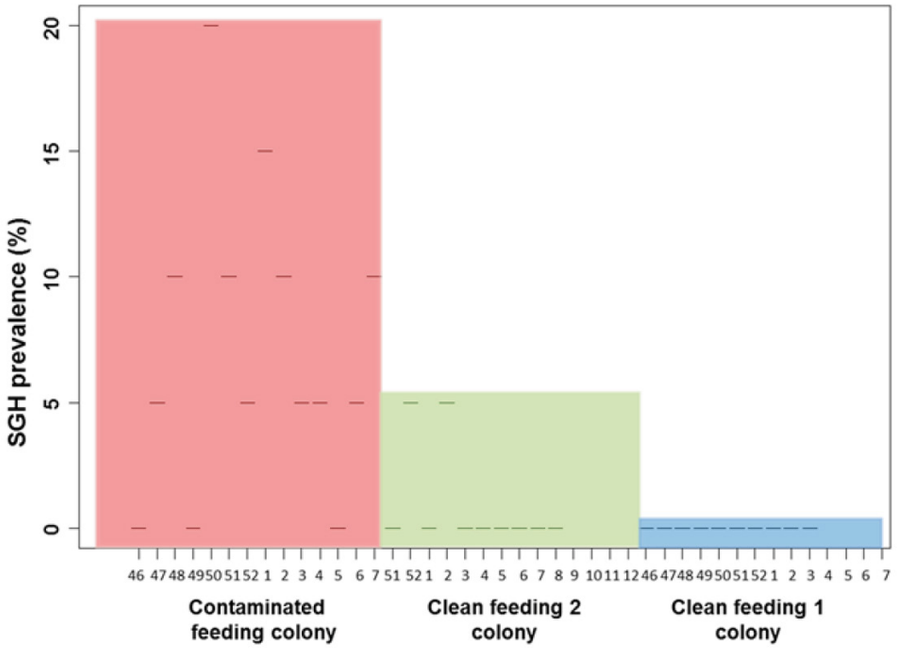

FIG. 1. Prevalence of SGH in tsetse colonies under different feeding conditions

Although these data are very promising for the development of virus management strategies, the clean feeding system cannot be used routinely in a large mass-rearing facility in view of excessive equipment and labour requirements. Clean feeding, however, could be used as an intervention strategy to reduce the initial virus load in a highly infected colony prior to the implementation of other management strategies to block virus transmission by inhibiting virus infection (e.g. neutralizing the virus infection by virus specific antibodies) or blocking virus replication (e.g. antiviral drugs or oligopeptides) when the colony is returned to the normal feeding system.

Antiviral drugs

The impact of the long term administration of two antiviral drugs (acyclovir and valacyclovir) on virus replication and fly productivity was assessed. The experimental flies were fed with blood that was heavily contaminated with the virus (the blood was used for feeding three cages with normal colony flies) and treated with 300 µg/mL of the two drugs before being offered to the flies. The experiment lasted for 16 months and the following results were obtained: (i) due to the low productivity of the flies treated with acyclovir, the treatment was terminated; (ii) flies treated with valacyclovir showed an acceptable level of productivity throughout the experimental period; (iii) dissection of the flies treated with valacyclovir showed a significant reduction in SGH as compared to non-treated flies; and (iv) the qPCR

results showed the presence of the SGHV in the valacyclovir-treated flies but at a significantly lower level than in the non-treated flies (Fig. 2).

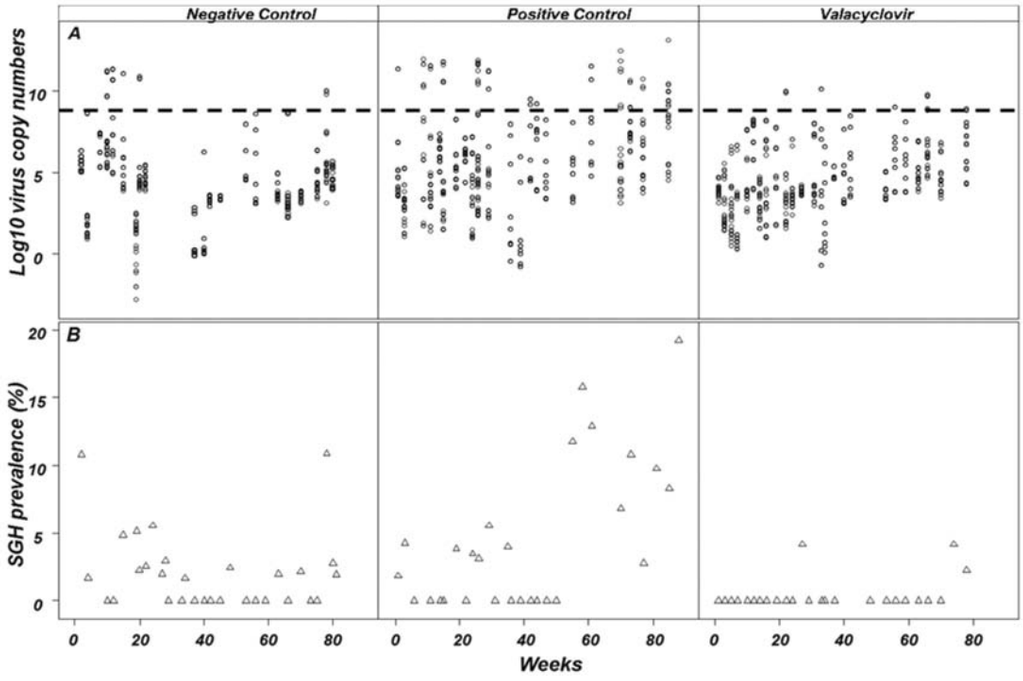

FIG. 2. Impact of valacyclovir treatment on (A) virus load and (B) SGH prevalence in a G. pallidipes *colony.*

Valacyclovir and clean feeding on SGH prevalence

The impact of a combination of treatments was assessed on the prevalence of SGH, i.e. (i) the selection of virus-negative teneral flies by non-destructive PCR; (ii) clean feeding; and (iii) valacyclovir treatment. Teneral flies were screened with non-destructive PCR and when found negative selected and divided into two groups: (i) the first group was fed on clean blood and designated as 'screen-clean feeding', and (ii) the second group was provided with blood mixed with valacyclovir (300 µg/mL) and designated 'screened–vala'. In addition, a second set of non-screened teneral flies were also divided into two groups: (iii) fed on clean blood and designated as 'non-screened-clean feeding'; and (iv) fed on blood mixed with valacyclovir and designated as 'non-screened-Vala'. The results indicate that combining pre-selection of flies, clean feeding and valacyclovir treatment resulted in the complete elimination of SGH from treated flies in less than 33 weeks, corresponding to approximately 2–3 generations. When the teneral flies were not screened for virus presence, clean feeding alone or in combination with valacyclovir treatment reduced but did not eliminate the SGH after 60 weeks (Fig. 3).

These results are quite important in case the colony manager — due to some difficulties to start a new colony from wild collected flies — needs to establish a new colony with a low virus load from an existing colony infected with SGHV. Our results indicate that combining pre-selection of flies for SGHV, clean feeding and valacyclovir treatment will significantly reduce the time needed to establish a new *G. pallidipes* colony that is free of symptomatic infections.

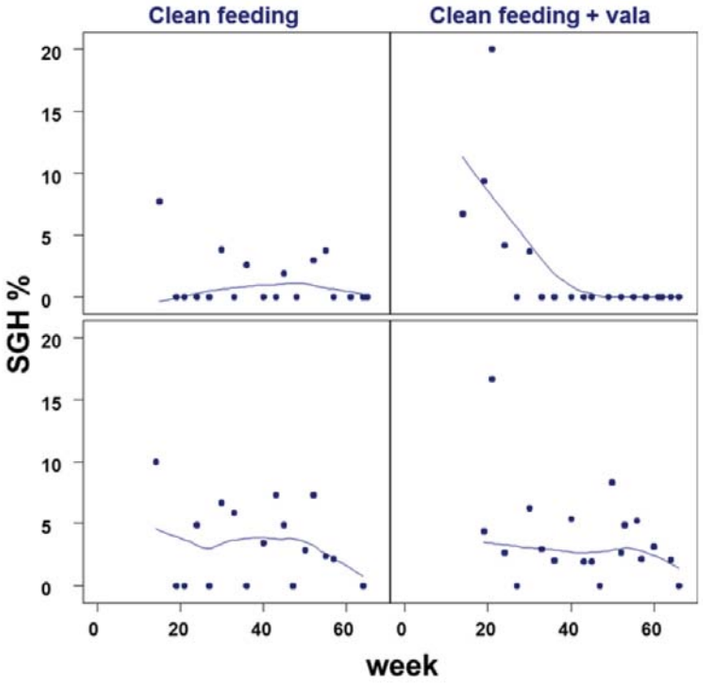

FIG. 3. Effect of the combination of clean feeding, screening flies with non–destructive PCR for virus infection (top graphs are screened and bottom graphs are non–screened flies) and valacyclovir treatment on the expression of SGH syndrome.

Neutralizing the virus infection with virus specific antibodies

Neutralizing virus infections with virus specific antibodies is a well known strategy to manage virus infections. Therefore, we tried to neutralize the virus released into the blood through incorporating virus specific antibodies in the blood meal. Six antibodies were prepared: (i) two antibodies against the p74 protein; (ii) one antibody against each of the proteins expressed by the open reading frames 10, 17 and 96 produced; and (iii) one antibody extracted from rabbits used for tsetse feeding. All of the antibodies were tested against GpSGHV virus particles by Western blot analysis and the results showed that five of the antibodies reacted specifically to the virus particles. The antibodies extracted from the rabbit were reactive against non-hypertrophied glands as well as purified virus particles, indicating the presence of antibodies to normal saliva components. Further experiments to optimize the use of virus specific antibodies in neutralizing the SGHV infection will be undertaken.

Effect of virus prevalence on the mating competitiveness of male Glossina pallidipes

An assessment was made of the mating competitiveness of *G. pallidipes* displaying SGH, the impact of SGHV infection on male *G. pallidipes* performance in terms of mating success and the mating behaviour of wild females mated with infected males. The results indicate that males with SGH were less successful in mating when competing with males without SGH. In addition, a high percentage of females mated with males with SGH had empty spermathecae. Although most of the females mated with males with SGH had empty spermathecae, these females did not show any increased tendency to remate with males with normal salivary glands.

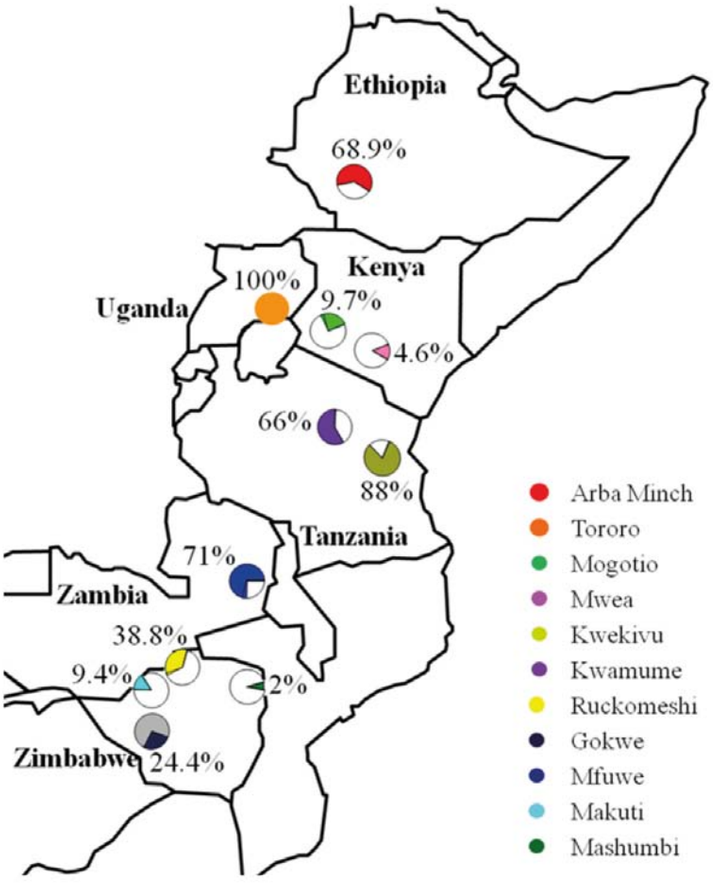

FIG. 4. Prevalence of Glossina *virus in wild populations of the tsetse fly* Glossina pallidipes. *Flies were obtained from six countries in eastern and southern Africa. Prevalence of the virus in respective geographical location is indicated in the pie charts.*

Prevalence and genetic variations of virus in wild tsetse populations

Whereas the prevalence of SGHV in laboratory colonies can be as high as 80%, the prevalence of the virus in wild *G. pallidipes* populations has been previously reported as low (0.4–2%). Since most SGHV infections are asymptomatic, molecular diagnostic tools may provide a more realistic picture of the virus epidemiology in wild fly populations. Therefore, we investigated the virus prevalence, and determined the number, frequencies, genetic distance within and between the virus haplotypes present in wild populations of *G. pallidipes*, and evaluated the feasibility of developing antiviral strategies in fly colonies based on these findings.

We used a total of 1 972 *G. pallidipes* samples collected from ten geographical regions in eastern and southern Africa. We used the *G. pallidipes* colony that has been maintained at the IPCL, Seibersdorf (originally from Tororo, Uganda) as the reference sample. On average, 34.08% of the samples were PCR-positive for SGHV. There were wide variations in virus prevalence, ranging from 2% (Mashumbi, Zimbabwe) to 100% (Tororo). Generally, fly samples collected from national parks had a relatively higher virus prevalence, e.g. Arba Minch (68.9%) and Luangwa (71%).

We performed phylogenetic and gene genealogy analyses of SGHV circulating within the fly populations using concatenated sequences of viral genes encoding for proteins thought to be involved in initial viral infections (p74, pif1, pif2 and pif3) and replication (dnapol). Phylogeny based on the concatenated sequences of the viral genes resulted in 23 virus haplotypes distributed among two main clades (albeit with poor bootstrap values). The reference haplotype was the most widely distributed.

The analysis revealed that although the prevalence of SGHV significantly varies from one geographical location to the other, genetic diversity of the virus is rather low, with random distribution among the virus haplotypes. It is, therefore, feasible to develop and apply common antiviral strategies in different laboratory colonies of *G. pallidipes* regardless of the virus haplotype in circulation.

This study needs to be complemented with an analysis of fly samples from central and western Africa to complete the picture of the virus epidemiology.

Development of strategies to impede virus entry and transmission using phage display library and oligopeptide bioassays

For successful infection of tsetse fly, the virus must attach to and eventually traverse the fly gut epithelium, and find its way into the salivary glands for horizontal transmission. This indicates that the fly midgut must have receptors that are specific to the epitopes on the virus surface. We, therefore, initiated preliminary trials to evaluate the potential of using a 12 amino acid oligopeptide (protein) to impede the attachment of the virus in the midgut of *G. pallidipes*. The virus load in the flies fed with the oligopeptide-supplemented blood meals showed reduction by at least two orders of magnitude compared to the controls.

Based on these promising findings, we are currently screening a random phage display library for peptides that will bind to brush border membrane vesicles derived from the midgut epithelia of *G. pallidipes*. The aim is to identify specific genes and sequences responsible for virus attachment to the midgut.

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.

We set up a series of bioassays that examined the impact of the antibiotic ampicillin on both asymptomatic flies and flies injected with infected gland extracts. Our working hypothesis was that the antibiotic-induced immune suppression would stimulate increased virus titre in flies and potentially trigger the expression of SGH symptoms. In light of the delayed effects of virus injections on tsetse flies, we followed all treatments through to the emergence of the F1 progeny.

Treatment of *G. pallipides* with filtered virus preparation did not cause any initial perturbations in adult activity or fitness. Injected adults during the initial four weeks mated, took blood meals, and produced a complement of F1 progeny that mirrored those produced by the control adults. Significantly, injection of the virus did not cause an increase in the degree of detectable SGH in the injected adults.

Feeding the *G. pallipides* flies ampicillin-augmented blood did not increase the virus titre in either male or female parents. The antibiotic treatment, however, had a profound impact on the microbial symbionts as evidenced by the elimination of *Sodalis* from the offspring of treated mothers. Our initial results suggest that this treatment decimates the bacterial symbionts; the implication is that the innate defence, if suppressed by this treatment, plays either no role or a minor role in regulating GpSGHV replication.

Preliminary PCR data on virus-injected *G. pallipides* has shown that virus titre increased about a hundred fold compared to the level observed in non-injected flies. The increase in titre appears to plateau and did not continue to increase with fly age. These observations suggest that the injected virus enters and replicates but is not able to spread to other adult tissues. The addition of ampicillin did not increase replication in the injected flies. It should be noted that research conducted on asymptomatic flies to date has been limited to qPCR analysis that provides data only on viral copies. Nothing is known about the replicative pathway of SGHV in asymptomatic tsetse flies. Potentially, the observed increase in viral copies represents only DNA replication; it may not reflect the production of infectious viral particles.

Fruit flies

Reproductive compatibility among Anastrepha fraterculus *populations of different geographical origin – roles of sexual isolation and chemical communication*

The South American fruit fly *Anastrepha fraterculus* (Fig. 5), originally thought to be a wide ranging species, seems actually to be composed of a complex of cryptic species differing in mating behaviour, host range and distribution. Data so far collected have suggested the existence of at least six species: *A.* sp. 1 *aff. fraterculus, A.* sp. 2 *aff. fraterculus, A.* sp. 3 *aff. fraterculus, A.* sp. 4 *aff. fraterculus, A. fraterculus* Mexican morphotype and *A. fraterculus* from Andean highlands and Venezuelan coastal lowlands.

FIG. 5: Anastrepha fraterculus *during mating experiments in field cages.*

In August–October 2011, the IPCL hosted three researchers from Argentina, Ms Clara Liendo, Ms Mariana Mendoza and Ms Teresa Vera, to continue the studies on the reproductive compatibility within the complex in order to allow quantifying the degree of isolation occurring among putative species. Knowledge on the mating compatibility among geographically separated populations, as well as the possible mechanisms involved, is important to determine the feasibility of sterile insect technique (SIT) implementation on a regional basis. Previous experience with other tephritid pests, such as the Mediterranean fruit fly (medfly), has shown that rearing facilities in different Member States can use the

same strain of fly. Moreover, establishing sound diagnostic tools to determine species limits and formal naming of these putative species will be critical for importing authorities in determining which of them may or may not be quarantine pests.

IPCL staff in collaboration with the Argentinian scientists focussed on the following activities: (i) to complete field cage mating compatibility tests in pairwise combinations of the following populations: Xalapa (Mexico), Piura (Peru), Vacaria, Piracicaba and Pelotas (Brazil) and Tucumán (Argentina); (ii) to collect the pheromones of the mentioned populations in order to characterize the pheromone profile by gas chromatography; and (iii) to continue studies on the re-mating behaviour of the populations from Argentina and Peru.

Mating compatibility tests (pre-zygotic isolation)

FIG. 6. Standard field cages in a climate controlled insect greenhouse.

Fly mating compatibility studies in field cages were carried out with populations originating from Jalapa (Mexico), Piura (Peru), Vacaria, Piracicaba and Pelotas (Brazil) and Tucumán (Argentina). The tests followed standard procedures and were carried out in a screened field cage containing a host tree that was deployed in a temperature controlled insect greenhouse (Fig. 6). Sexual isolation was observed in the Piura–Pelotas, Piura–Vacaria, and Piracicaba–Tucuman combinations (index of sexual isolation >0.50), whereas the index of sexual isolation of the Tucuman–Jalapa, Pelotas–Jalapa and Piracicaba–Jalapa combinations remained below 0.24, indicating a tendency of random mating.

Female re-mating behaviour

Re-mating tests were conducted using populations from Peru and Argentina. For each mating combination, one cage was set up under no choice conditions, resulting in four treatments: Peru males x Peru females; Argentina males x Argentina females; Peru males x Argentina females; Argentina males x Peru females. Copulating pairs were collected and mating time, latency time, etc. were carefully recorded. Mated females were offered a second mating opportunity after two days with sexually mature virgin males of the same origin.

Re-mating rate was determined as the percentage of females that copulated twice. Mating pairs involving Argentina females showed a shorter latency to mate and longer copulation duration than mating pairs involving Peru females. The refractory period (time elapsed between the first and second copula) was similar for the four combinations. Re-mating rates were different among the mating combinations, with a tendency towards higher re-mating rates when Argentina females were involved, irrespective of male origin.

The differences in latency and copula duration found before in previous studies were confirmed in this study. The tendency with respect to the refractory period and the re-mating rate showed that the Argentina strain is more polygamous than the Peru strain. Taking the evidence together, females seems to exert the control in the variables measured.

Pheromone analysis

Volatiles from calling males (those that emit pheromones to attract the female flies) were collected using a published methodology. Collections were made in greenhouses under ambient environmental conditions at the IPCL. The samples have been sent to the Centre for Medical, Agricultural and Veterinary Entomology (CMAVE), Gainesville, Florida where they will be analysed chemically in collaboration with Patricia Fernández (INTA, Argentina) and Peter Teal (USDA, USA).

Quality evaluation and mating competitiveness of new marker strains of the Mediterranean fruit fly Ceratitis capitata *under semi-mass rearing conditions.*

Monitoring of released and wild flies in the target area is a very important, and expensive, component of any SIT programme. The information obtained through trapping in the field is required as an essential decision making tool for the field operations. Based on the trapping results, the programme manager controls the release of sterile insects and/or the application of additional control measures.

For this, it must be possible to distinguish wild and released flies. Several internal or external marking systems are available, e.g. the use of fluorescent dye powder in tsetse and fruit fly SIT programmes, and the use of internal dyes (e.g. calco red) in Lepidoptera release programmes. Most of these marking schemes have several disadvantages, i.e. concerns about worker safety, contamination of equipment and the environment, unreliability of the dye in the field, and the very high labour cost associated with the examination of trapped insects. Various, more reliable and less costly alternatives exist, ranging from the utilization of rare mitochondrial haplotypes to introducing additional visible mutations, either through classical genetics or through molecular biology. Some genetic markers, as represented by visible mutations, are available but they are all associated with some level of reduced fitness of the insect. Using a mitochondrial DNA (mtDNA), it has been possible to incorporate a marker into medfly genetic sexing strains (GSS). However, this has only been possible because there is very extensive knowledge available on the worldwide variation of the mitochondrial genome of this species. This required level of information is not available for other pest species and is unlikely to be developed. However, a genetic transformation approach removes this requirement by being able to introduce a unique piece of DNA into the genome. This unique piece of DNA can either be monitored using PCR or can be used to express a fluorescent protein that can be monitored using the appropriate microscope. The latter is obviously the most efficient in analysing large numbers of trapped insects.

A GSS, based on the VIENNA 8 strain, was developed for the medfly that carries two independent fluorescent markers; one red fluorescent marker for the whole body and a green fluorescent marker for the sperm (VIENNA 8 – 1260) (Fig. 7). The availability of a sperm marker opens the additional possibility to determine the mating status of trapped wild females, i.e. to determine whether the females were mated by a released, sterile or a wild male. Without the ability to make this distinction, the detection of a single mated and, therefore, potentially fertile female in the field can lead to very costly additional control measures (additional releases, etc.). The double marked medfly GSS was evaluated for one year at standard small scale as well as medium scale level. Parameters determined include egg hatch, pupal recovery

and adult recovery. The overall productivity was reduced as compared to the original GSS VIENNA 8 without the molecular markers. However, at this point, it cannot be excluded that this reduction is linked to a lack of genetic diversity in the strain as its construction required single pair crosses. In addition, the strain was monitored with respect to the occurrence of exceptional phenotypes which would indicate a problem with stability. However, that was not the case.

As a follow-up, another trial was initiated in 2011 at the IPCL to assess the productivity, stability and mating competitiveness of these new marker strains of the medfly under semi-mass rearing conditions. The strains that were evaluated are (i) the standard VIENNA 8 GSS that is currently used in most mass-rearing facilities in the world for eradication and suppression programmes; (ii) the VIENNA 8 'Sergeant' strain that carries a morphological mutation that is expressed as three abdominal stripes; and (iii) the VIENNA 8 '1260' strain. All strains carry the *tsl* (*temperature sensitive lethal*) mutation which is lethal to the female flies when incubated at higher temperatures. The overall quality of the strains was evaluated using procedures as described in the FAO–IAEA–USDA manual for quality control of insects produced for use in the SIT. The egg production, egg hatchability, egg to pupa recovery, pupal weight adult emergence and flight ability, and male:female ratio were measured at each generation, starting from F3. Furthermore, the irradiation effects (100 Gy from a ^{60}Co source) on male and female flies from the three strains were measured as expressed in egg fertility. The results showed a high hatchability rate in control groups for all three strains, and a very high (>99%) and total sterility (100%) for irradiated males and females, respectively.

The field cage experiment (using F3 generation flies) indicated that the marker strains *Sr* and 1260 were competitive to a satisfactory degree (RSI values : 0.26, 0.22 and 0.35 for VIENNA 8, Sergeant and 1260, respectively), and that their mating latency and copulation duration were similar to those of their wild-type counterparts for all three strains.

Development of a cold storage treatment for peach fruit fly Bactrocera zonata *(Diptera: Tephritidae)*

In 2010, the United States Department of Agriculture (USDA) and the FAO/IAEA signed an agreement on 'The Development of Phytosanitary and Regulatory Treatments for Exotic Tephritid Fruit Flies', which was extended in 2011. The agreement solidified the collaboration between the IPCL and the USDA to develop, among others, a cold storage treatment for the peach fruit fly *Bactrocera zonata* (Saunders) (Fig. 8). This pest poses a significant trade barrier for the export of fruit from infested countries such as Egypt into the USA. Originally native to south and south-east Asia, *B. zonata* is known to infest a variety of fruits including peach, mango, guava, papaya and citrus. There is currently no USDA–APHIS approved cold treatment for *B. zontata* that will allow the import of fruit from countries where it is known to be established. Recent detections of *B. zonata* in California (2006) and Florida (2010) highlight the importance of establishing reliable treatment schedules for imported fruit to protect the fruit growing regions in the USA.

FIG. 8. Adult Bactrocera zonata

Cold treatment can be an effective means to eliminate the risk of introduction for many tropical fruit flies as they are well tolerated by many fresh fruit commodities and are often compatible with standard industry practices. In addition, they are capable of being implemented during overseas transit periods.

The aim of this project was to develop a cold treatment schedule to be used for in-transit shipments of citrus from regions where *B. zonata* is known to occur. The objectives were to first compare the cold tolerance of third instar *B. zonata* with that of two species with well developed cold treatment schedules, namely the medfly and the Mexican fruit fly *Anastrepha ludens* (Loew). Experiments used navel oranges infested with the three species maintained at 1.7°C. If the results of the results of the first objective were insufficient, to then conduct confirmatory treatments with more than 30 000 *B. zonata* larvae in infested fruit to validate the efficacy of the treatment with a sufficient degree of confidence.

The fruit used for all experiments were navel oranges imported from Egypt. Trays with fruit were placed into cages and exposed to adults of each species for 30–120 min depending on the age and number of adult flies available. Fruit was then stored in temperature controlled environmental chambers at 26°C to allow eggs to hatch and larvae to develop to third instar, i.e. the most cold-tolerant stage for each of these species, before the start of the cold treatment.

Treatment groups were placed into a 1 965 L capacity environmental chamber (Thermotron Model SE2000–4, Holland, MI), customized with an internal glass door with four 18 cm access ports to allow access to the items in the chamber without opening the doors (Fig. 9).

FIG. 9. Environmental chamber used for the cold treatment of Bactrocera zonata.

The results indicated that the A. *ludens* model was significantly different from both *B. zonata* and *C. capitata*, but no differences in the models of *C. capitata* and *B. zonata* were detected. Since the lack of significant difference in cold tolerance between *C. capitata* and *B. zonata* did not provide clear evidence that *B. zonata* was less tolerant to cold treatment, additional confirmatory tests were conducted at 18 d cold treatment. In the 18 d confirmatory tests, a total of 36 820 B. zonata larvae were treated in infested navel oranges with no survivors. These experiments, therefore, provide strong evidence that an 18 d cold storage treatment of oranges or other citrus varieties that may be a host with temperatures not exceeding 1.7°C will safely mitigate the risk of introducing *B. zonata* into the USA.

Mosquitoes

Research in the mosquito group focussed on the two disease vector species *Anopheles arabiensis*, an important vector of malaria, and *Aedes albopictus*, an important vector of dengue fever and Chikungunya.

Radiation studies with Aedes albopictus *from Reunion Island*

Ae. albopictus males from Reunion Island were completely sterilised in a ^{137}Cs gamma irradiator with a dose of 40 Gy, which is consistent with similar studies using a ^{60}Co gamma and X ray irradiator with the Rimini strain of the same species. After a period of rest, a second mating induced the same level of sterility in females indicating that there was no recovery of fertility.

The effects of irradiation on the rotation cycle of the genitalia of *Ae. albopictus* was studied with un-irradiated males and males irradiated with 35 Gy as pupae. The overall impact of irradiation on the rotation cycle was minor and irradiation did not negatively affect the rotation of the male genitalia in *Ae. albopictus*.

Mating studies of sterile and wild male Aedes albopictus *on Reunion Island*

Competitiveness tests are fundamental in assessing the quality of sterile males that are to be released in the context of AW–IPM programmes with an SIT component. These studies have to be carried out in an environment as close as possible to natural conditions to obtain a reliable indicator of competitiveness. Experiments were conducted in semi-field conditions on Reunion Island (Fig. 10) where 35 Gy irradiated male *Ae. albopictus* competed against wild males for mating with wild females for 7 d.

FIG. 10. Field cage trials on Reunion Island to assess competitiveness of sterile versus wild Aedes albopictus *males (left); a sterile and wild male* Aedes albopictus *competing for a wild female (right).*

The competitiveness index (CI) (which can range from 0–1) was low (0.15) when males were released on the day following emergence at a S:W male ratio of 1:1. The CI values increased to 0.59 and 0.82 when males were kept in the laboratory for 5 d before being released. We suggest that competitiveness in irradiated males was enhanced either by age or by the time spent under relaxed conditions in the laboratory before release.

Mating success of sterile Aedes albopictus *males*

Four day old male *Ae. albopictus* irradiated with 40 Gy were as successful as un-irradiated males in inseminating females. However, when the males were given ten females every day for 15 d, the insemination success of irradiated sterile males was lower as compared to those that were un-irradiated. The daily mean number of females inseminated by a sterile male was always lower compared to the mean number of females inseminated by un-irradiated males. *Ae. albopictus* females had mostly only one spermathecae filled when inseminated by sterile males, whereas females mated by un-irradiated mosquitoes had 1–3 spermathecae filled.

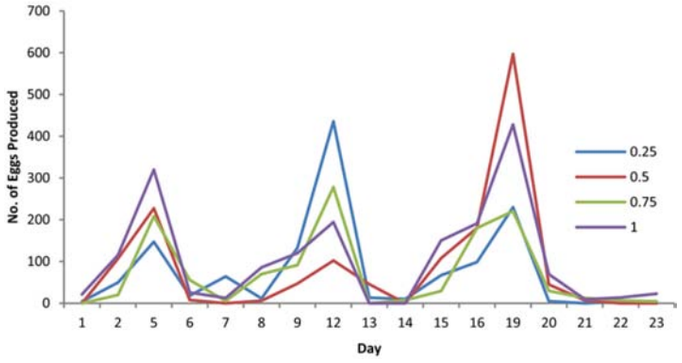

FIG. 11. Mean number of eggs produced per day for the four treatments: 0.25, 0.5, 0.75 and 1.0 (proportion of male to female). Periods of high egg production were different for each treatment but overall egg production was similar.

Effect of male:female ratio in mass-rearing cages on egg production of Aedes albopictus

In a mass rearing facility, there is a need to reduce the male:female ratio in colony cages to free up extra males for sterilization and releases. Whereas this approach could alleviate some of the 'nuisance' effect of males attempting to mate with unreceptive females, it should, however, not adversely affect egg production.

Standard laboratory cages filled with male:female (100 females) ratios of 0.25, 0.5, 0.75 and 1.0 had increased male mortality at the 0.25 sex ratio, suggesting an increase in male competition and multiple mating resulting in increased energy expense and mortality. Total egg production was not different for any of the treatments, indicating that a ratio of 0.25 males to females is adequate to ensure that enough females are inseminated to maintain egg production. A repeat of this trial with higher female densities (1000 females) but using the same male:female ratios again resulted in similar egg production, irrespective of the sex ratio.

A comparison of three artificial diets for the rearing of Aedes albopictus

The Centro Agricoltura Ambiente (CAA) in Bologna, Italy was recently awarded the status of an IAEA Collaborating Centre, and some research carried out at the IPCL on optimizing mass rearing practices for *Aedes albopictus* has been in direct support of the experimental releases programme in Bologna. Larval diet is of critical importance as it has a major influence on the produced adult mosquitoes and three mosquito-specific diets were tested for the rearing of *Ae. albopictus:* (i) the CAA diet consisted of ground cat food (Friskies®), brewer's yeast and fish food (Tetramin®); (ii) the FAO/IAEA diet consisted of a mixture of equal parts of bovine liver powder and tuna meal with a small addition of vitamin mix (the diet was originally developed at the IPCL for the rearing of *Anopheles arabiensis*); and (iii) the new FAO/IAEA diet was developed specifically with the needs of *Ae. albopictus* in mind and consisted of a mixture of tuna meal, bovine liver powder, squid liver powder, brewer's yeast and a small addition of vitamin mix.

Larvae fed the FAO/IAEA and new FAO/IAEA diets had significantly shorter larval periods and a significantly larger proportion of the total number of males appearing within the first

24 h of pupation than those fed with the CAA diet. Survivorship and size as measured by wing length did not differ significantly across diet types. It is, therefore, recommended to rear *Ae. albopictus* larvae on the new FAO/IAEA diet.

Digital estimation of egg production of Aedes albopictus *under mass rearing conditions*

Egg production, a commonly used indicator of female fecundity and the quantification thereof is integral to the standardization of any mass rearing operation. Without accurate information as to colony productivity, any predictions regarding the availability of mosquitoes for release, experimentation or for colony re-stocking purposes are potentially inaccurate, unreliable and a possible waste of time, materials or funds. To date, egg quantification has involved manually counting eggs under a microscope, a laborious and time-consuming process.

A new method for quantifying small quantities (<500) of Aedine eggs, originally developed at the University of Kentucky, USA, was refined, recalibrated and adjusted to oviposition papers more indicative of those expected in mass rearing operations (>10 000 eggs per paper). The digital estimation of eggs on oviposition papers is a two-step process whereby the papers are first scanned using a digital scanner and then analysed using ImageJ. The image is converted to greyscale and any pixels not containing eggs are digitally subtracted. The subsequent pixels are analysed for their total area which will be used to create an equation used to estimate egg numbers. To digitally remove all background elements excluding eggs, the threshold of the image must be adjusted, a process in which an arbitrary limit (threshold) is set at which pixels with intensity values lower than this threshold will appear as black (eggs) and those with a value greater than the threshold will appear as white (oviposition paper, shadows, legs and scales).

A series of threshold values were used to develop equations for egg estimation and these resulting equations were further subjected to validation tests in which the accuracy of their estimations was tested. A threshold value of 140 was chosen as its estimations were closest to a line with perfect estimation (i.e. slope = 1) (Fig. 12).

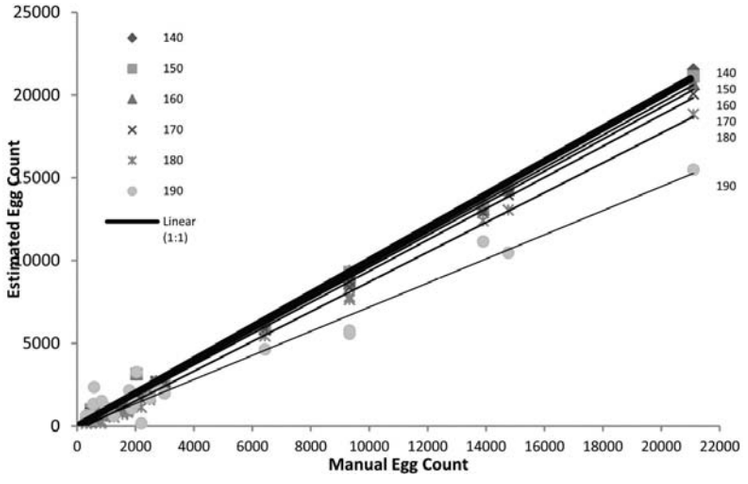

FIG. 12. Relationship between manual egg counts and estimated egg counts for each threshold as compared to a line of perfect estimation (bold). Linear regressions for each data set are shown.

Sperm production of Anopheles arabiensis *with respect to genetic manipulation, dieldrin application and irradiation*

Before their release, *An. arabiensis* males undergo several harsh treatments. First, a GSS (Ano IPCL1) was created from the original wild type strain that is based on a dieldrin resistant mutation using a translocation. Second, treating Ano IPCL1 eggs with dieldrin will completely eliminate all female mosquitoes, mostly at the L1 stage. The absence of female larvae and pupae in the rearing process reduces costs, space and labour requirements. Finally, before being released, the male pupae need to be irradiated with a dose of 70 Gy. The individual and combined effects of the genetic manipulation, dieldrin treatment and irradiation on sperm number and sperm production in male *An. arabiensis* were investigated.

Two day old Dongola (wild type) and Ano IPCL1 males had a similar number of mature sperm cells and sperm production increased with age. The irradiation and dieldrin treatments reduced the initial sperm stock and there was no further sperm production for the irradiated males; on the contrary, the number of sperm decreased with age. Although the dieldrin treated mosquitoes had a lower number of sperm in their initial stock, sperm production increased with age. In addition, sperm production in dieldrin-treated/irradiated males was higher on day 6 as compared to males that were only irradiated. This would suggest that the dieldrin treatment, aimed at eliminating females, might have an unexpected radiation protectant effect on *An. arabiensis* germinal cells.

This observation, if confirmed, could be very useful for a control programme integrating the release of sterile males allowing the production of sterile males with an adequate sperm complement. However, it needs to be ascertained whether this sperm production is possibly accompanied with a recovery of fertility. Moreover, with respect to SIT, the effect of lower quantities of the initial sperm complement of sterile males on mating ability, as measured by the amount of sperm transferred per female and the number of females inseminated, must also be assessed.

Effect of continuous and intermittent access to sugar before blood feeding on Anopheles arabiensis *female egg production and survival*

The availability of sugar is important to females as a nutrient source for survival, flight and fecundity. However, sugar is known to have a negative effect on the avidity of females to a blood meal which results in reduced fecundity. Female mosquitoes that continuously had access to sugar produced fewer eggs than mosquitoes that had intermittent access to sugar. There was no difference in survival of males and females between treatments. Sugar deprivation before blood feeding enhanced cage egg production by 50% compared to continuous sugar delivery, without impairing male and female survival.

Anopheles arabiensis *pupae management: pupa stage duration and pupa cold storage*

To minimize somatic damage due to irradiation, pupae must be irradiated as close as possible to emergence. However, to develop adequate protocols, pupa duration and emergence dynamics must be known. In addition, in some countries such as Sudan, pupae are irradiated at the production site, before being transported to the field site(s) for release. Transport from the production site to the release sites could take up to 6 h by truck, and pupae could start

emerging during the journey. To prevent emergence during transport, the development of the pupae could be delayed by storing the pupae at low temperatures. The effects of cold storage on pupa duration, adult male mosquito survival and male reproductive abilities were studied.

Pupa development time

The mean duration of the pupal stage of *An. arabiensis* under standard rearing conditions was 28 h and 35 min. Aside from the intrinsic duration of the pupa stage, the great variation in pupa duration was probably due to the fact that 80% of the mosquitoes emerged the following day during the artificial dusk period, irrespective of the time at which larvae pupated the previous day. This is most likely related to the decreasing light intensity in the laboratory that mimics the dusk that triggers the emergence between 1900 and 2000 h. This information is very useful for the management of the pupae and for the development of adequate irradiation schemes, e.g. emptying the trays in the morning and collecting all the pupae at the end of the day, and irradiating them the next day just before dusk will ensure an optimal sterilisation with limited damage. Irradiated pupae can then be transported to the release site or be stored in climatic chambers.

Pupae cold storage

Groups of mature larvae were exposed in dark climatic chambers to 16°, 12° or 10°C for 6 h. Pupae maintained at 16°C started emergence during the cold storage period whereas the time of emergence was delayed for pupae maintained at 10° and 12°C. There was no significant difference in mortality for the four treatments and the total number of eggs produced by females mated with males which had been chilled for 6 h at 12°C was similar to egg production of females mated with control males. Storing pupae for 6 h at 10°C deteriorated the mating abilities of the male mosquitoes.

These results seem to indicate that storing *An. arabiensis* pupae for 6 h at 12°C sufficiently delays adult emergence without negatively affecting viability or reproductive ability. More experiments are needed to assess the impact of chilling pupae on the competitiveness of the males. In addition, it needs to be assessed whether the cumulative effect of the other treatments given to the male mosquitoes (dieldrin treatment and irradiation) negatively affects adult male competitiveness.

*Effects of dieldrin treatment at larval stage on adult male longevity (***An. arabiensis***, IPCL1 strain)*

The effect of dieldrin treatments of larvae of the GSS of *An. arabiensis* (IPCL1) on adult male longevity was assessed. The analysis showed that the treatment with dieldrin did not affect the longevity of the IPCL1 males. The assessment of any effects on the quality of adult males destined for release is important for all treatments the adults must endure prior to release. The effects of treatment with dieldrin at the egg stage on adult male longevity will also be tested using the same general protocol.

CAPACITY BUILDING

In 2011, the IPCL hosted ten cost-free experts, six consultants, three interns, two PhD students and six fellows, the latter funded by the Department of Technical Cooperation in the following area:

1. Mating compatibility studies with members of the *Bactrocera dorsalis* and *Anastrepha fraterculus* complex to entangle their taxonomic status to facilitate trade

   Cost-free experts

   Mark Schutze (Australia – 2 x 3 months)

   Anthony Clarke (Australia – 3 months)

   Adalecio Kovaleski (Brazil – 6 months)

   Clara Liendo (Argentina – 5 weeks)

   Mariana Mendoza (Argentina – 6 weeks)

   Ihsan Ul Haq (Pakistan – 9 months (funds from USDA))

   Consultant

   Teresa Vera (Argentina – 4 weeks)

2. Rearing of fruit flies and protocols to sterilise flies using the X ray machine as an alternative to gamma rays

   Cost-free expert

   Hernan Fernando Donoso Riffo (Chile – 2 weeks)

   Consultant

   Gustavo Taret (Argentina – 6 months

3. Tsetse mating studies in field cages and developing pupal transport methods in support of the tsetse eradication programme in the Niayes of Senegal

   Cost-free expert

   Gratian Mutika (Zimbabwe – 12 months (funds from US PUI))

Consultant

Idrissa Kabore (Burkina Faso – 12 months)

4. Post-harvest cold treatment of fruit fly infested fruits to facilitate trade of fruit commodities in fruit fly infested countries to the USA

Cost-free expert

Guy Hallman (USA – 2 weeks)

5. Developing mass rearing techniques and the SIT package for disease transmitting mosquitoes

Interns

Odessa Madakacherry (USA – 12 months

Arianna Pugglioli (Italy – 3 months)

PhD student

Clelia Oliva (France – 6.5 months)

Consultants

David Damiens (France – 12 months)

Fabrizio Balestrino (Italy – 9 months)

6. Fruit fly genetics to develop better marker strains

Intern

Wang Huiji (China – 11 months)

7. Developing management strategies for the tsetse virus in support of the tsetse eradication project in Ethiopia and other East African countries

Cost-free expert

Drion Boucias (USA – 3.5 months)

PhD student

Henri Kariithi (Kenya – 8 months)

Consultant

Adel Barakat (Egypt – 3 months)

Furthermore, six Technical Cooperation fellows were trained in the following areas:

M. Ababe (Ethiopia – 1 week – tsetse rearing)

N. N. Yin (Myanmar – 1 month – fruit fly rearing, mating studies)

G. Ouedraogo (Burkina Faso – 1.5 months – tsetse rearing),

F. Ahmed (Sudan – 1 month – mosquito rearing),

L. Nyakupinda (Zimbabwe – 1 week – tsetse fly rearing),

S. Al-Shuwaili (Iraq – 3 months – fruit fly rearing).

SERVICES

The IPCL continuously receives requests from collaborators in coordinated research projects, in technical cooperation projects, and from universities and research institutes for the supply of biological material.

In 2011, the IPCL supplied 25 350 tsetse fly pupae (*G. pallidipes*, *G. p. gambiensis* and *G. brevipalpis*) to six research institutes in Slovakia, Germany, Switzerland, the United Kingdom, Italy and the USA.

In addition, the IPCL supplied 11 ml of eggs, 1500 (45 ml) pupae and 53 ml of adults of the olive fly *Bactrocera oleae*, 15 ml of eggs, 45 ml of larvae and 1200 ml of pupae of VIENNA 8 *Ceratitis capitata*, 1.5 ml of eggs, 8 ml of larvae and 250 ml of pupae from other *Bactrocera* spp. and 20 ml of pupae of *Anastrepha fraterculus* South American fruit fly. The fruit fly pupae were delivered to 13 research institutes in France, Portugal, Brazil, Greece, Slovakia, Italy, Malaysia, Chile, Turkey and the USA.

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PLANT BREEDING AND GENETICS LABORATORY

EXECUTIVE SUMMARY

The Plant Breeding and Genetics Laboratory (PBGL) is an integral part of the Plant Breeding and Genetics Section and contributes to the goals of supporting capacity in Member States in the use of induced mutations for sustainable food security. The PBGL achieves these goals through adaptive research and development, including the development and transfer of protocols and guidelines for the efficient use of mutations and associated biotechnologies for crop improvement, capacity development and service.

Increasing security restrictions are imposed on the shipment of gamma emitters, and the production and refurbishment of such sources. This limits the use of gamma rays for plant mutation induction. X ray irradiation is an alternative method for plant mutation induction and in 2011, the PBGL initiated work to adapt and optimize commercial X ray machines for seed and in vitro plantlet irradiation. Adaptors developed by the PBGL for efficient irradiation in machines employing orbital rotation have been used successfully for mutation induction in seven seed crops and three vegetatively propagated crops.

Research and development activities at the PBGL in 2011 have focused on developing efficient mutation detection methods with the acquisition of new equipment for mutation discovery and characterisation at the DNA level. This included an increase in capacity via an equipment donation from the Fred Hutchinson Cancer Research Center and Howard Hughes Medical Institute in Seattle, Washington, USA, and the initiation of a new next generation sequencing facility. In addition, positive control kits for mutation discovery were distributed to four Member States. The development of a new seed phenotyping facility was also initiated to strengthen capacity in phenomics. Protocols, guidelines and information were released to Member States though the IAEA web site and through 17 publications. Programmatic activities in research and development were supported through 13 external collaborations and partnerships.

The PBGL has been actively involved in two coordinated research projects (CRPs) on: (i) enhancing the efficiency of induced mutagenesis through an integrated biotechnology pipeline; and (ii) molecular tools for quality improvement in vegetatively propagated crops including banana and cassava. In 2011, results from these CRPs were published in 11 international, peer reviewed scientific journals, plus four national reports.

In the area of human capacity building, nine fellows from nine countries were trained for a total of 36 months. Five scientific visitors were hosted at the PBGL. Two interns also received training in the laboratory in 2011.

Requests for irradiation services increased in 2011; 29 requests were received from 19 Member States for over 20 plant species. Most requests were for gamma irradiation, but four Member States specifically requested X ray irradiation.

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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Human population growth is projected to reach a staggering 9 billion people by 2050. Who will feed the growing billions? How can more food be produced on less land? How can we protect crop yields from climate change, pests and diseases? These are major issues of our generation. In the past, mutation breeding has played a major role in improving crops and stabilizing food security concerns worldwide. Classic examples are the improved yields brought about by semi-dwarf mutant varieties of wheat and rice in the mid-twentieth century, which created the 'green revolution'. Mutation induction via irradiation is an established method in providing useful variation for crop improvement, but the sought after mutant is a rare event. Today, these mutants can be detected efficiently using high throughput phenotyping and genotyping methods. It is these enabling technologies that have provided increased activity in mutation induction as exemplified by an increase in the number of countries requesting PBGL mutation induction services compared to 2010. Research and development activities at the PBGL in 2011 have focussed on developing efficient mutation detection methods with the purchase of

new equipment for mutation discovery and characterisation at the DNA level. The development of a new seed phenotyping laboratory was also initiated.

All the work of the PBGL is driven by Member State demands. The PBGL has a mandate to carry out: (i) research and development, (ii) training and (iii) laboratory services directed at mutation induction, mutation identification and the take up of promising mutants by plant breeders. Success is dependent upon building links with practical plant breeders in developing countries, for which phenotyping is seen as a major bridge between PBGL activities and Member State breeding programmes. In 2011, the PBGL was re-structured to strengthen activities in phenotyping and to develop high-throughput phenotypic screens for mutant trait selection. In breeding terms, plant phenotype relates to expressed traits, particularly those that provide improved field performance. The primary targets are yield and yield stability, but other traits include quality, pest and disease resistance, tolerance to abiotic stresses and agronomy. Post-harvest traits include handling, post-harvest loss, storage and processing. Traits of concern to Member States are a major driver for our work at the PBGL.

Traditionally, the PBGL has focused on three tropical crops: rice, banana and cassava. Although these crops continue to be important in our research and development activities, there has been a shift of focus from crops to traits. This shift reflects new challenges and demands of Member States. For example, global wheat production is now threatened by new biotic (e.g. Ug99 rust disease) and abiotic (e.g. drought and flooding) stresses for which solutions are urgently required and these must be reflected in the PBGL activities. Therefore, in addition to our main tropical glasshouses which house rice, cassava, banana and sorghum, the PBGL has a temperate glasshouse, growth chambers and field plots where a greater range of crop species may be grown (including wheat, barley, maize, sorghum and tomato). Mutant traits under research and development include salt tolerance in rice and wheat, seed quality in rice and sorghum, drought and pathogen tolerance in banana, storage and starch quality of cassava tubers and fodder quality in barley.

Mutation induction – enhancing mutation induction using X rays

Mutations may be induced in plants by exposure of their propagules, such as seeds, meristematic cells, tissues and organs, to physical and chemical mutagens. Gamma irradiation has been one of the most effective and commonly used physical mutagens for plant mutation breeding. Over 3200 mutant crop varieties have been released worldwide, the majority of which have been developed through gamma irradiation (http://mgvs.iaea.org/). However, increasing worldwide security restrictions imposed on the shipment of gamma emitters, and the production and refurbishment of such sources now severely limits the use of gamma rays for plant mutation induction. The PBGL has therefore initiated work to adapt and optimize commercial X ray machines (e.g. the RS-2400) for seed and in vitro plantlet irradiation. The RS-2400 uses orbital rotation and rotation along the axis of the sample canister to ensure even irradiation of samples. Plant samples, therefore, need to be packed and fixed in a central location to ensure uniform irradiation. Adaptors have, therefore, been developed for seed and in vitro samples (Fig. 1). These adaptors have been used successfully for mutation induction in seed crops (rice, sorghum, lupin, barley, wheat, artemisia and jatropha) and vegetatively

propagated crops (cassava, ginger and banana) and X ray mutagenesis was requested by four Member States in 2011 for five crop species (see Services below).

FIG. 1. PBGL scientists have developed a range of specialized adaptors for irradiation of seed and tissue culture materials using X ray equipment employing orbital rotation around an X ray source.

Mutation detection – genotyping

The PBGL has continued to invest in research and development activities for the discovery and characterization of induced mutations in plants. The ability to recover and quantify the density and spectrum of induced mutations serves two main purposes. First, an accurate count of the type and number of mutations induced in a treatment allows a probabilistic estimation of how many mutant plants should be screened in order to have a good chance of recovering plants with the desired enhanced trait. Secondly, efficient recovery and targeting of induced mutations within genes and regulatory regions allows reverse genetics approaches, whereby only plants harbouring interesting mutations need to undergo rigorous phenotypic characterization. In many cases, this can mean reducing the number of plants evaluated by one or more orders of magnitude. In conjunction with coordinated research project D24012 'Enhancing the Efficiency of Induced Mutagenesis through an Integrated Biotechnology Pipeline', the PBGL has, for the past several years, focused on enzymatic mismatch cleavage based technologies for mutation discovery. The laboratory has developed optimized protocols (http://mvgs.iaea.org/LaboratoryProtocols.aspx) for both higher and lower-throughput mutation discovery, and has developed a positive control kit that is distributed to Member States upon request. In 2011, this kit was distributed to Austria, Bulgaria, the Syrian Arab Republic and the Philippines.

With the use of fluorescence based DNA analysers, enzymatic based mutation discovery methods have been optimized to the point where higher density mutations can be routinely recovered from thousands of individual mutagenized plants per day. Throughput in the PBGL has been limited, however, because the laboratory has operated only one such DNA analyser that is shared between research and development and capacity building activities. In 2011, the PBGL was pleased to receive the very generous donation of seven fluorescent LI-COR DNA analysers and one 384 channel liquid handling pipettor from the USA. The equipment was donated by the Laboratory of Dr. Steven Henikoff of the Fred Hutchinson Cancer Research Center and Howard Hughes Medical Institute in Seattle, Washington. With an estimated value of approximately US $400 000, the donation allows the PBGL to concomitantly expand research and development and training activities in the areas of mutation discovery and reverse genetics (Fig. 2). The PBGL used fluorescent based mutation screening in 2011 to optimize strategies for mutation of in vitro cultures of banana using isolated shoot apical meristems. This on-going work may provide a method to greatly reduce tissue culture manipulations and, thus, time and money when developing mutant banana populations and those of other facultative and obligate vegetatively propagated crops.

FIG. 2. Scientists from Member States are trained on mutation discovery techniques using equipment donated from the USA.

While enzymatic mismatch cleavage methods are advantageous because of their relatively low cost, and ease of use in laboratories with limited molecular biology infrastructure, the methods are not well suited for applications where

induced mutation densities are below approximately one event per million base pairs, or the mutation spectrum includes larger deletions and chromosomal aberrations. Such densities and spectra can be induced better by treatment of plant seed and tissues with varying dosages of gamma and X rays. Therefore, alternative methods are required. The most promising suite of approaches to emerge in the past several years is 'next generation sequencing' (NGS) technologies. At the 2010 research coordination meeting for CRP D24012, CSI Thomas Tai of the USDA, ARS in Davis California described pilot experiments for mutation discovery involving restriction phased genomic DNA libraries and Illumina paired-end NGS sequencing. Based on successes reported for recovery of high density point mutations in rice, the PBGL established a collaboration with the Laboratory of Professor Luca Comai of the UC Davis Genome Center whose group developed this methodology known as RESCAN (restriction enzyme-phased illumina sequencing libraries). In 2011, pilot experiments were initiated to evaluate the effect of low density gamma irradiation on in vitro cuttings of cassava. Previous efforts to study this gamma irradiated population of cassava using enzymatic methods required over 12 months of PBGL research and development activities. This work involved DNA extraction, quantification and concentration normalization from leaves from 3 000 mutagenized plants, development of gene-specific primers, sample arraying and mutation screening assays. The result was a mutation density estimation of approximately one event per 25 million base pairs. The pilot work using the RESCAN approach was used to confirm this estimation in approximately half the time using only ten samples. We expect the time for mutant evaluation to drop dramatically as this pilot phase involved method development and optimization for library preparation (wet bench experiments) as well as development of bioinformatics pipelines.

NGS technologies have clearly emerged as the cutting edge for DNA variant analysis. In addition to providing methods for the rapid estimation of mutation density and spectrum, NGS allows a comprehensive evaluation of natural nucleotide diversity (re-sequencing) that is useful for a range of breeding approaches. Additionally, methods have been developed for efficient reverse genetics, referred to as TILLING by sequencing. As such, the PBGL is committed to these approaches to assist Member States in the efficient use of induced mutations for sustainable crop intensification. In 2011, the PBGL was honoured with a major capital investment award of €365 589 for the establishment of an NGS facility. Most activities in 2011 were focused on equipment evaluation and procurement plans, with installation and trainings scheduled for 2012. This marks a major improvement for the PBGL, and once fully implemented, enhanced protocols and guidelines for the use of induced mutation for improvement of seed and vegetatively propagated crops should provide important efficiency gains for Member States engaged in mutation breeding projects.

Mutation detection – phenotyping

A new initiative at the PBGL in 2011 was to begin the development of seed phenotyping facilities. High throughput phenotyping (often referred to as 'phenomics') has been identified as being a bottleneck in plant genetics and breeding. Whereas high throughput genotyping, and especially current sequencing capabilities, can provide tens of thousands of data points, our ability to phenotype remains poor. Seed phenotyping is of particular interest to the PBGL as seeds are not only a major planting material, but are the harvestable products of many major

crop species, notably cereals (wheat, rice and maize) and oilseeds, beans, etc. Seeds also have an advantage in being easily transported. The seed laboratory is being equipped with non-destructive imaging systems that measure seed shape, size and colour and internal morphology. This work is augmented by collaboration with the University of Natural Resources and Life Sciences, Tulln, Austria which has expertise in the use of near infrared spectroscopy to detect altered seed composition. In addition, soft X ray imaging is being investigated to detect mutant seed phenotypes. These methods act as a pre-screen for seed quality and enable mutant candidates (present at low frequency) to be selected from large populations. These can then be subject to verification tests.

CAPACITY BUILDING

Individual fellowship training

Name	Country	Duration	Training topics
Saraye, Banumaty	Mauritius	4 months	Genetic diversity in chilli and tomato from Mauritius. TILLING and ecotilling of chilli and tomato germplasm including putative mutants.
Kyaw, Moe Thida	Myanmar	4 months	Induced mutagenesis for crop improvement. Phenotyping for nutrition traits in mutant rice lines.
Zaarawi, Wajida	Iraq	3 months	Mutation induction in sorghum. Screening for salt tolerance in wheat.
Dhouibi, Raouia	Tunisia	6 months	Genetic diversity and mutation induction in olive.
Kimno, Stephen Kipchirchi	Kenya	5 months	Mutation induction in Artemisia. Germplasm characterisation.
Maman, Maman Badamassi	Niger	4 months	Induced mutation and mutation detection using molecular characterisation.
Rabefiraisana, Harimialimalala J.	Madagascar	4 months	Induced mutation and mutation detection using molecular characterisation.
Diedhiou, Ibrahima	Senegal	3 months	Induced mutation and mutation detection using molecular characterisation.
Peloewetse, Elias	Botswana	3 months	Induced mutation and mutation detection using molecular biology techniques.

Scientific visitors

Brown, Julia	Jamaica	5 days	Induced mutation in crop improvement and other enabling technologies.
Nowbuth, Rita Devi	Mauritius	5 days	
Ali, Mohamed Ahmed	Sudan	10 days	
Stoilov, Lubomir	Bulgaria	5 days	
Jouhr, Mahfoudh	Syrian Arab Republic	10 days	

Interns

Kozak, Kamila	Poland	11 months	Cytological and phenotypical analyses of M1, M2 and M3 rice plants; technology transfer to the *Lupinus* spp.
Shirazi, Farzaneh	Islamic Republic of Iran	6 months	TILLING, ecotilling and molecular marker strategies for mutant germplasm characterisation.

SERVICES

Requests for radiation services increased in 2011; 29 requests were received from 19 Member States for over 20 plant species. Most requests were for gamma irradiation, but there were specific requests for X ray irradiation (*). Details are given below.

Irradiation services

Member State	Crop species
Germany	Ornamental plants and barley
Jamaica	Yam and ginger*
Poland	Lupin*
Mauritius	Brassica vegetables and banana
The Former Yugoslav Republic of Macedonia	Wheat and barley
Iraq	Wheat and sorghum
Namibia	Bambara groundnut and marama beans
Kenya	Barley*and artemisia*

Member State	Crop species
Tunisia	Olive
USA	Sugar beet
Spain	Calla lily
Nigeria	Cowpea
Botswana	Groundnut, cowpea and maize
United Kingdom	Wheat and cowslip
Senegal	Jatropha*, groundnut
Czech Republic	Brassica vegetables, *Ballontinica antipoda, Harmisiodaxa brevipes*
Oman	Barley and wheat
Uganda	Wheat
Jordan	Barley and wheat

Protocols, guidelines and information released in 2011

Screening for mutant genes and traits is a major activity of the PBGL. Protocols developed in 2011 for genotyping include: methods for accurate DNA quantification suitable for mutation discovery using free image analysis software; those for phenotyping include: a hydroponics seedling salt tolerance screen in rice. The PBGL protocol collection along with guidelines and information are given below and are available at http://mvgs.iaea.org/LaboratoryProtocols.asp (Table accurate on 13 April 2012, contents subject to change).

Title	Document type
Training manual: Molecular characterization of mutant germplasm	The PBGL's main training manual used in training courses.
Example data and data sheets for low cost DNA quantification	PBGL generated control image of genomic DNA to use for training on image analysis software. Standardized data sheets with auto-fill features for DNA quantification using known standards.
Mutation induction for plant breeding 101	Presentation on the basics of mutation breeding.
Radiation sensitivity tables	Guidelines for chronic and acute dosages of radiation for a variety of different crop species.

Title	Document type
SMTA	Standard material transfer agreement for sharing germplasm under the International Treaty on Plant Genetic Resources for Food and Agriculture.
TILLING and ecotilling protocols	A suite of protocols validated by the PBGL that cover all aspects of TILLING and ecotilling including primer design, sample normalization, arraying, mutation discovery, data analysis, and reporting and best practices for sample handling and nomenclature.
Positive control for mutation discovery using agarose gels, version 2.4	Protocol for mutation discovery using crude enzyme extracts and agarose gel electrophoresis.
Positive control for mutation discovery using LI-COR and agarose gels, version 2.5	Protocol for mutation discovery using crude enzyme extracts and fluorescence detection and denaturing polyacrylamide gel electrophoresis.
User feedback form, mutation discovery positive control kit, version 1.1	Feedback form for the mutation discovery kit.
Seedling hydroponics test for salt tolerance in rice	Protocol for screening rice for response to increasing salinity using a hydroponic growth system.

PUBLICATIONS

Czyczylo-Mysza, I., Marcinska, I., Jankowicz-Cieslak, J., Dubert, F. (in press). The effects of ionizing radiation on vernalization in winter wheat grains. Acta Physiologiae Plantarum.

Jain, S.H., Till, B.J., Suprasanna, P., and Roux, N. (2011). Mutations and Cultivar Development in Banana. Chapter 10. In Banana Breeding: Progress and Challenges; CRC Press, pp. 203–218.

Jankowicz-Cieslak, J., Huynh, O.A., Bado, S., Matijevic, M. and Till, B.J. (2011). Reverse-genetics by TILLING expands through the plant kingdom. Emirates Journal of Food and Agriculture 23(4):290–300.

Jankowicz-Cieslak, J., Huynh, O., Matijevic, M., Bado, S., and Till, B.J. (in press). TILLING and Ecotilling approaches to study biotic and abiotic stress responses in Musa. 9th Plant Genomics European Meeting (Plant GEM) meets global challenges, 4–7 May, 2011, Istanbul, Turkey (abstract only).

Jankowicz-Cieslak, J., Kozak, K., Till, B.J., Galek, R. and Sawicka-Sienkiewicz, E. (in press). Ecotilling as efficient SNP discovery tool to investigate genetic variation in *Lupinus* sp. Proceedings of the 13th International Lupin Conference, 6–10 June, 2011, Poznan, Poland (abstract only).

Jankowicz-Cieslak, J., Brozynska, M., Rapacz, M., Huynh, O., Matijevic, M., Bado, S., and Till, B.J. (in press). TILLING and Ecotilling approaches to study genetic contributions to drought response in Musa. Abstract book of the International Conference on Plant Gene Discovery Technologies, 23–26 February, 2011, Vienna, Austria (abstract only).

Jankowicz-Cieslak, J., Brozynska, M., Cieslak, J., Adu-Gyamfi, J., Mba, C., Till, B.J., Rapacz, M. (2011). Physiological Profiling Of Drought Response in Diverse Musa Accessions. In: Plant and Animal Genomes XIX Conference, San Diego, USA, 15–19 January 2011, Abstract P669.

Kozak, K., Jankowicz-Cieslak, J., Bado, S., Till, B.J., Galek, R. and Sawicka-Sienkiewicz, E. (in press). Inter-varietal differences of *Lupinu*s sp. responses to the chemical and physical mutagens. Proceedings of the 13th International Lupin Conference, 6–10 June, 2011, Poznan, Poland (abstract only).

Kozak, K., Jankowicz-Cieslak, J., Bado, S., Till, B.J., Galek, R. and Sawicka-Sienkiewicz, E. (in press). Inter-varietal differences of *Lupinus* sp responses to the chemical and physical mutagens. Proceedings of the 13th International Lupin Conference 6–10 June, 2011, Poznan, Poland (abstract only).

Kozak, K., Jankowicz-Cieslak, J., Bado, S., Till, B.J., Galek, R., Sawicka-Seinkiewicz, E. (in press). Optimization of TILLING and Ecotilling techniques in Lupinus sp. In: International Conference on Plant Gene Discovery Technologies, 23–26 February, 2011, Vienna, Austria (abstract only).

Lokko, Y., Mba, C., Spencer, M., Till, B. and Lagoda, P, (2011). Nanotechnology and synthetic biology — potential in crop improvement. Journal of Food, Agriculture & Environment 9(3&4):599–604.

Maghuly, F., Jankowicz-Cieslak, J., Calari, A., Ramkat, R., Till, B.J. and Laimer, M. (2011). BMC Proceedings, 5 (Suppl. 7):O50.

Maghuly, F., Jankowicz-Cieslak J., Till, B., Calari, A., Ramkat, R. and Laimer, M. (in press). Ecotilling as efficient SNP discovery tool to investigate genetic variation in non-domesticated Jatropha curcas. Abstract book of the International Conference on Plant Gene Discovery Technologies, 23–26 February, 2011, Vienna, Austria (abstract only).

Till, B.J., Jankowicz-Cieslak, J., Huynh, O., Bado, S., and Matijevic, M. (in press). High-throughput TILLING in Vegetatively Propagated Plants. International Conference on Plant Gene Discovery Technologies, 23–26 February, 2011, Vienna, Austria (abstract only).

Till, B.J., Huynh, O., Dussoruth, B., Jankowicz-Cieslak, J. (2011). Reverse-Genetics and Polymorphism Discovery In Musa. In: Plant and Animal Genomes XIX Conference, San Diego, USA, 15–19 January 2011, Abstract W071.

Till, B.J., Jankowicz-Cieslak, J., Huynh, O., Bado, S., Matijevic, M. (2011). Mutation Induction and Reverse-Genetic Strategies for Understudied Crops. In: Plant and Animal Genomes XIX Conference, San Diego, USA, 15–19 January 2011, Abstract W281.

Swanston, J.S., Middlefell-Williams, J.E., Forster, B.P. and Thomas, W.T.B. (2011). Effects of grain and malt β-glucan on distilling quality in a population of hull-less barley. Journal of the Institute of Brewing 117:389–393.

EXTERNAL COLLABORATIONS AND PARTNERSHIPS

Institution	Topic
International Center for Tropical Agriculture (CIAT), Cali, Colombia	Induction and detection of mutation events in South American cassava lines for enhanced productivity and competitiveness through value addition
International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria	Induction and detection of mutation events in African cassava lines for enhanced productivity and competitiveness through value addition
International Rice Research Institute (IRRI), Manila, Philippines	Induced mutations in rice for tolerance to abiotic stresses (including salinity)
International Network for the Improvement of Banana and Plantains (INIBAP), Bioversity International, Montpellier, France	Induced mutations in *Musa* for tolerance to biotic stresses and development and deployment of genomics tolls for the crop
Austrian Institute of Technology, Health & Environment Department (Dr Silvia Fluch, Dr Kornel Burg), Tulln, Austria	Gene expression profiling in drought stages
University of Natural Resources and Life Sciences (Dr. Hans Vollmann), Tulln, Austria	NIRS analysis in characterising mutant seed phenotypes
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 under-studied crops

Institution	Topic
University of Natural Resources and Life Sciences, Department of Biotechnology (Dr Theresa Scharl), Vienna, Austria	Statistical data evaluation
Agri-Science Queensland (Prof Jerome Franckowiak), Hermitage Research Facility, 604 Yangan Road, Warwick, Australia	Barley crossing protocol; barley mutants and mutation breeding
The James Hutton Institute (Dr. William Thomas), Invergowrie, Dundee, Scotland, United Kingdom	Barley crossing protocol; barley genetic stocks, genetic markers for low lignin mutants
Nordic Genetic Resource Center (Dr Udda Lundqvist), Alnarp, Sweden	Classic barley mutants, mutant gene descriptions and nomenclature
University of California Davis Genome Center (Prof Luca Comai and Dr Isabelle Henry), Davis, California, USA	Developing next generation sequencing strategies for discovery of induced mutation events in genomes of vegetatively propagated crops

SOIL AND WATER MANAGEMENT & CROP NUTRITION LABORATORY

EXECUTIVE SUMMARY

The Soil and Water Management and Crop Nutrition Laboratory (SWMCNL) has been actively involved in research and development, capacity building, technology transfer and quality assurance in 2011. The focus of the SWMCNL's work is to develop, adapt and deliver a range of soil, water and crop management technology packages to Member States using isotopic and nuclear techniques to identify factors and processes that affect the interactions between soil, water and farming systems. The main areas of activity in pursuit of these objectives are applied research and development, technology transfer, isotopic analyses to Member States where analytical facilities are not currently available, and development of guidelines on the use of nuclear and isotopic methods in soil and water management and crop nutrition.

For research and development in support of the coordinated research project (CRP) D1.50.12 on 'Soil Quality and Nutrient Management for Sustainable Food Production in Mulch-based Cropping Systems in Sub-Saharan Africa', the SWMCNL initiated a series of greenhouse, field based and laboratory activities that aim to develop a new generation of isotopic techniques that can assist Member States in the rapid and cost effective assessment of carbon distribution between above and below-ground plant parts, the effectiveness of land management strategies in enhancing soil organic carbon (SOC) accumulation and storage, and the quantification of the stability of the stored SOC. A protocol that uses low cost N_2O gas sampling chambers inserted into the ground was also developed to quantify the extent of N_2O emissions under different land uses and management practices, as part of support to CRP D1.50.12. The protocol can be readily adapted by Member States for an N_2O emission study.

The SWMCNL was also involved in the evaluation of the use of compound-specific stable isotopes (CSSI) as markers to identify hot spots of land degradation for effective soil conservation measures. Through close collaboration with the National Institute of Water & Atmospheric Research (NIWA) in Hamilton, New Zealand, CSSI was found to complement the information on soil erosion rates provided by fallout radionuclides under Austrian and east Slovenian agro-ecological conditions. These findings and the protocols developed will support CRP D1.20.11 on 'Integrated Isotopic Approaches for an Area-wide Precision Conservation to Control the Impacts of Agricultural Practices on Land Degradation and Soil Erosion'.

The SWMCNL also continued development of a simple and rapid technique for routine $^{18}O/^{16}O$ extraction of water from plant and soil samples, as part of CRP D1.20.09 on 'Managing Irrigation Water to Enhance Crop Productivity under Water-limiting Conditions: A Role for Isotopic Techniques'. The methodology developed by the SWMCNL is simple, fast, affordable and portable, also compared with a commercially available immersion cooler, or with cooling by liquid nitrogen and dry ice; the new methodology is valuable in quantifying the removal of water from the soil around crop roots through soil evaporation and transpiration. This information will be used to identify soil and water management practices that minimize water losses via soil evaporation.

The SWMCNL was also actively involved in the IAEA Scientific Forum on 'Water Matters: Making a Difference with Nuclear Techniques', which was held on 20–21 September 2011 during the 55th IAEA General Conference. A series of displays of promotional material illustrated the work of the SWMCNL on agricultural water management, including a drip irrigation prototype mimicking the operation of a drip system in the field, a live demonstration of the vacuum distillation set-up and a demonstration of root development in large transparent plastic tubes. Sixteen impact reports from both CRP and technical cooperation projects involving the SWMCNL were developed and distributed during the Forum. The SWMCNL also conducted training activities to support technology transfer under various technical cooperation projects. Two fellows, three scientific visitors and one intern received hands-on training for three months, each in the use of isotopic techniques in agricultural water management, fertilizer use efficiency, biological nitrogen fixation and nitrogen balance, and soil degradation assessment.

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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Testing innovative CSSI techniques to identify hot spots of land degradation in Austria

A new stable isotope technique, using CSSI signatures of organic markers in the soil, can identify hot spots of land degradation to complement the information on soil erosion rates provided by fallout radionuclides. Knowing where to focus erosion management efforts ensures efficient and cost effective soil conservation strategies to reduce soil loss and improve the sustainability of arable land.

The technique is based on the concept of land use being defined by the plant community growing on the land. These plant communities label the soil where they grow by exudating organic materials that act as markers (e.g. fatty acids). Although all plants produce organic materials, the stable isotopic signature in these materials is different for each plant species.

This technique, originally adapted by NIWA in Hamilton, New Zealand, is now being tested in ten countries under CRP D1.20.11. This technique has also been tested under Austrian agro-ecological conditions in a small watershed located in Mistelbach, 60 km north of Vienna. Soil samples from different representative land uses (e.g. pasture, maize, sugar beet, wheat) in the watershed were collected and the ^{13}C signatures of fatty acids in the soil determined. The compound specific stable isotope results showed that 28% of accumulated sediments in the lower watershed came from agricultural fields used for the cultivation of sugar beet, whereas 68% came from drainage channels (under pasture) at the bottom of the studied watershed. Around 4% of the sediment originated from the maize and mixed crop rotation land. These results show that the CSSI technique is a powerful tool for identifying critical hot spots of land degradation in agricultural landscapes.

A protocol for nitrous oxide (N_2O) measurement

FIG. 1. Low cost gas chamber for measuring nitrous oxide gas.

Greenhouse gas (GHG) emissions to the atmosphere and their contribution to climate change have become important concerns worldwide. Intensification of agricultural activities, such as the increased use of synthetic nitrogen fertilizer, inefficient use of irrigation water, and application of farm effluents and animal manure to croplands and pastures can be the source of nitrous oxide (N_2O) emissions from farm lands. A protocol using low cost N_2O gas sampling chambers (Fig. 1) for measuring nitrous oxide (N_2O) emission, a GHG, was developed in the SWMCNL as part of the CRP D1.50.12. This protocol will allow the quantification of the extent of N_2O emissions under different land uses and management practices that will help reduce the GHG emissions.

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

In 2011, in support of CRP D1.50.12, the SWMCNL initiated the development of a new generation of isotopic techniques that can assist Member States in the rapid assessment of carbon distribution between above and below-ground plant parts, and the quantification of the stability of the stored SOC. Data obtained could further be used to improve the effectiveness of land management strategies in enhancing SOC accumulation and storage.

The SWMCNL implemented two major activities to develop and adapt these innovative isotopic techniques.

Testing of an analytical technique for assessing soil organic carbon stability

This technique, which is based on the use of ^{15}N natural abundance analysis of critical soil organic matter pools, has been tested in an arable soil poor in SOC (Cambisol) and was shown to have very good reproducibility. Further testing on soils at the upper end of the SOC scale is currently underway. A protocol is expected to be produced in early 2013.

Developing an isotope based technique for assessing the long term contribution from different crops to soil organic carbon sequestration in complicated crop rotation systems

A long term field experiment focusing on conservation agriculture has been used. The experiment was implemented 16 years ago on fertile soils rich in SOC (Chernozem) at the research station of the University of Natural Resources and Life Sciences Vienna (BOKU) in Gross-Enzersdorf, Austria. Data on SOC stocks and bulk ^{13}C isotope analyses are now available for three contrasting treatments of the experiment, i.e. conventional and zero-tillage under a cereal based cropping system (mainly wheat), and permanent grassland (alleys), at different soil depths to 1 m (Fig. 2). Higher carbon stocks were observed under conservation agriculture, with a significant difference in the upper soil layers. Besides the importance of deep soil sampling for calculating carbon stocks and assessing carbon distribution, the first results also indicated the high repeatability of the isotopic analyses. By measuring bulk isotope signatures of the possible sources of SOC, the contribution of grass versus wheat to SOC in the upper soil could be determined. The preliminary results also suggest that fatty acids may be used to estimate more precisely the contribution from different crops to SOC sequestration in complicated crop rotation systems.

Two further activities: (i) assessing short term SOC accumulation and storage in poor soils in simple crop rotation systems at the AGES (Austrian Agency for Health and Food Safety) research station of Grabennegg; and (ii) testing isotope based techniques under tropical cropping systems in the SWMCNL greenhouse at Seibersdorf, are in progress.

Combined use of fallout radionuclides, naturally occurring radionuclides and stable isotopes in soil erosion assessment

In soil erosion assessment using fallout radionuclides, one of the major challenges is finding an undisturbed reference site. Through the combined use of fallout radionuclides (^{137}Cs) and stable ^{15}N and ^{13}C isotopes, the procedure for selecting reference sites could be optimized. Preliminary results from East-Slovenia showed that stable isotopes can assist in identifying sites that were not disturbed by agricultural practices. In South East Spain, background radionuclide information (^{137}Cs and naturally occurring radionuclides) established for undisturbed sites allowed a better assessment of the recent impact of agricultural activities on the magnitude and extent of soil degradation linked to olive tree cultivation.

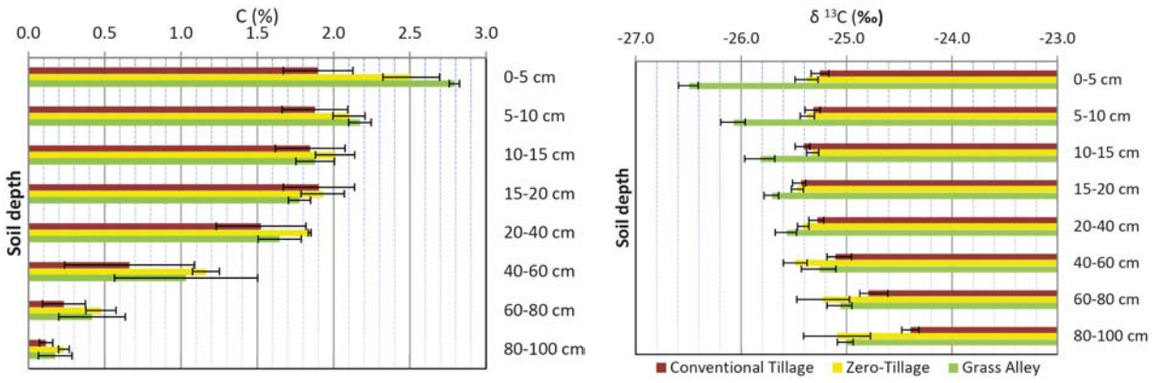

FIG. 2. SOC content and ^{13}C signatures for different depths under conventional and zero-tillage, and grass alleys at the long term experiment at a site in Gross-Enzersdorf (BOKU University). Error bars denote standard deviations.

Agricultural water management

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

As part of an investigation of crop water use efficiency, the SWMCNL developed a simple, fast, affordable and portable vacuum-distillation set-up and methodology for extracting water from soil and plant samples for isotopic analysis (Fig. 3). This set-up greatly simplified the quantification of water removal from the soil around crop roots through soil evaporation and transpiration. The information is useful for identifying soil and water management practices to minimize water losses via soil evaporation.

A major modification and improvement made in 2011 was the connection of commercially available sample storage tubes (15 mL glass culture tubes with GL18 screw caps) directly to the distillation unit without the need to transfer sample to other containers. The set-up can also accommodate more than 24 samples for distillation at any one time; hence, large numbers of samples can be extracted within a short time period. A comparison was also made using an immersion cooler instead of dry ice and liquid nitrogen, so that the methodology can be adapted to suit Member State facilities, as the immersion cooler can be obtained commercially. The preliminary results showed that full recovery (extracting more than 99% of the total water

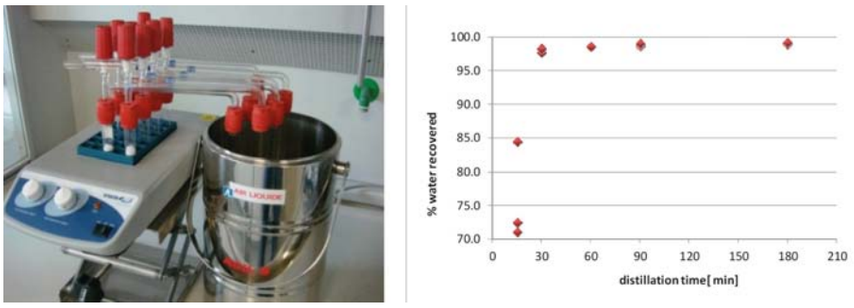

FIG. 3. Distillation of samples (left) and % water recovery versus distillation time of a clay soil (right).

in the samples) and reproducible isotopic ratios could be reached after 30 min of distillation time for both sandy and clay soils using the immersion cooler. The improved water extraction methodology provides a greatly enhanced and streamlined analytical capability which has always been a bottleneck in the past.

IAEA Scientific Forum on Water Matters: Making a Difference with Nuclear Techniques

The SWMCNL and the SWMCN Section were both involved in the IAEA Scientific Forum on 'Water Matters: Making a Difference with Nuclear Techniques' (Fig. 4), which took place from 20–21 September 2011 during the 55th IAEA General Conference. This forum was to promote the joint efforts of Member States and other international organizations with the IAEA in key water issues, by informing high level conference participants of the numerous and highly successful cooperative projects in IAEA Member States in the fields of agricultural water management, water resource assessment and aquatic pollution control, which are the three key pillars of water activities in the IAEA.

FIG. 4. The ADG of FAO, Ms Ann Tutwiler, delivered a speech during the Scientific Forum (left).

Drip irrigation prototype

FIG. 5. Drip irrigation prototype displayed during the Scientific Forum (right).

As part of the Scientific Forum, the SWMCNL developed a drip irrigation prototype (Fig. 5) mimicking the operation in the field to demonstrate and create awareness of the effectiveness of saving water and improving water use by crops. This technology can be applied with fertilizer through a process called fertigation. Many participants at the Forum were enthusiastic about adapting this technology for their countries.

Interactive maps

Two interactive maps on the percentage of total agricultural land that is irrigated and the percentage of total fresh water withdrawals allocated to agriculture were created using StatPlanet browser based interactive data visualization and mapping application. The maps allow the instantaneous, interactive capability of exploring differences in fresh water withdrawal and the percentage of irrigated land with just a mouse-click. These maps can be accessed at the SWMCN agricultural water management webpage http://www-naweb.iaea.org/nafa/soils-water.html.

In the week preceding the Forum and during the water forum, the above mentioned vacuum distillation set-up for extracting plant and soil samples, and the drip irrigation prototype were displayed. In addition, the SWMCN subprogramme organized a display of promotional

material to illustrate the work of the Section. A demonstration of root development in large transparent plastic tubes was also mounted.

ANALYTICAL AND QUALITY ASSURANCE SERVICES

In 2011, a total of 5304 plant and soil samples were analysed by the SWMCNL for CRP and technical cooperation projects in Member States, as well for in-house applied research and development.

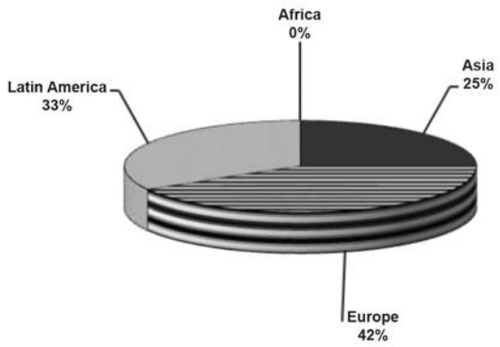

FIG. 6. Regional distribution of participating countries.

Ten stable isotope laboratories participated in the annual proficiency test (PT) on ^{15}N and ^{13}C isotopic abundance in plant materials, jointly organized by the University of Wageningen, Netherlands and the SWMCNL. The regional distribution is shown in Fig. 6. Seven out of ten laboratories reported acceptable ^{15}N data and six out of seven laboratories reported ^{13}C data within the control limits. Two ^{15}N data sets were wrongly reported resulting in very high z scores.

CAPACITY BUILDING

Technical support

The SWMCNL provided technical support to nine technical cooperation projects in the areas of soil fertility, erosion and sedimentation and biological nitrogen fixation. In addition, the acting Head of the SWMCNL, Mr Joseph Adu-Gyamfi, provided support as the scientific secretary to the CRP on 'Selection and Evaluation of Food (Cereal and Legume) Crop Genotypes Tolerant to Low Nitrogen and Phosphorus Soils through the Use of Isotopic and Nuclear Related Techniques' (D1.50.10).

The final RCM for this CRP was held in November 2011 at the IAEA in Vienna. The main conclusions were:

- Seedling screening tools demonstrated significant genotypic variation for root traits.
- Cultivars identified with some of these traits proved superior for uptake of P and N under conditions of nutrient stress.
- In a number of cases cultivars with superior growth, nutrient acquisition and efficiency obtained good yields of grain under conditions of nutrient stress.
- Positive agro-ecological outcomes were identified that are related to the performance of cultivars selected for favourable root traits.

- Nuclear tools, specifically the use of ^{15}N and ^{32}P as tracers proved valuable in studies that sought physiological explanations for superior genotype performance.

- The genotypes identified in a number of cases provide valuable resources for plant breeding programmes aimed at enhancing P and N use efficiency in crops.

- The CRP has created a database on how cereal and legume crops can acquire N and P in low nutrient soils. This database will be further expanded and interpreted using multivariate analysis.

Fellows and fellowship training

Therese Monette Nourice (Seychelles)

Julius Sesay (Sierra Leone)

Although there were only two fellows in 2011, they received hands-on training evaluating methods of fertilizer application (fertigation versus soil application) on the growth and yield of maize and soybean. They were also trained in the use of soil water monitoring equipment (such as the neutron probe, Diviner 2000 capacitance sensors and tensiometers) for scheduling irrigation. This training will help in their technical cooperation projects to formulate best soil and water management practices.

Scientific visitors

Dr Robert Schwartz, Soil Scientist from the Soil and Water Management Research Unit, USDA–ARS, Bushland, TX, USA, 6 April 2011. Dr Schwartz has been working on soil and water related topics (e.g. soil water measuring techniques, conservation agriculture) both within the USA and in developing countries.

Dr Vesna Zupanc of the Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Slovenia, 7 October 2011 to present a short seminar and to discuss the dataset of stable isotopes (^{13}C and ^{15}N) analysed from a joint field experiment with the SWMCN Laboratory team carried out in East Slovenia.

Ms Ann Tutwiler, FAO–ADG, Rome on 19 September 2011 for an update on the current activities of the SWMCN laboratory.

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