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Laboratories

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CONTENTS

ANIMAL PRODUCTION AND HEALTH LABORATORY

EXECUTIVE SUMMARY

Livestock is one of the fastest growing agricultural sub-sectors in many countries and there is a growing demand for livestock products, primarily driven by fast growing economies in Asia and Africa. Demand for livestock products in 2050 is expected to nearly double in South Asia and Sub Saharan Africa although there is a huge imbalance in the level of consumption across different regions. Huge differences in productivity exist between livestock in Asia/Africa and industrialized countries. Improving livestock production in these regions is a big challenge due to several reasons that include lack of high genetic merit animals with high producing ability, small holder production systems with limited resources, tropical vectors and animal diseases. Nevertheless, livestock production is becoming increasingly intensified worldwide resulting in increased interaction of human, livestock and wildlife. This has resulted in increased incidence of zoonotic diseases like Ebola and influenza in the recent times. According to the FAO report on "World Livestock 2013: Changing Disease Landscapes", seventy percent of the new diseases that have emerged in humans over recent decades are of animal origin and, in part, directly related to the human quest for more animal-sourced food. Added to this, globalization and climate change are redistributing pathogens, vectors and hosts that result in changes in the human and animal disease landscapes.

Another major effect of increased intensification of animal production systems is the growing threat to livestock biodiversity. In the report on the State of the World's Animal Genetic Resources for Food and Agriculture, FAO shows that approximately one livestock breed has been lost every month during the last decade alone. Livestock populations have evolved over centuries due to sustained natural and artificial selection with adaptation to local agricultural production systems and agro-ecological environments. The domestic animal genetic resource represents a unique source to respond to the present and future needs of livestock production. Loss of such unique and precious genetic resources is a huge concern for sustainable livestock production and future global food security. To address these issues and to support IAEA and FAO Member States in overcoming these challenges, major activities of Animal Production and Health Laboratory (APHL) are focused on two primary areas: (i) control of transboundary animal diseases through the development and transfer of tools related to diagnosis, molecular epidemiology and preventive vaccination and (ii) improving the genetic potential of local livestock breeds to increase productivity and conservation of livestock biodiversity through the effective implementation of the Global Plan of Action for Animal Genetic Resources in Member States.

During 2015, one of the major focuses of APHL was to support Member States in tackling the bird flu crisis in Western and Northern Africa. From January to April 2015, a re-emergence of the deadly Highly Pathogenic Avian Influenza H5N1 strain (HPAI-H5N1) occurred in Western Africa. On 16 January 2015, Nigeria confirmed the re-emergence of HPAI-H5N1 to the World Organization for Animal Health (OIE). The disease spread rapidly reaching 18 of the 37 states in the country, decimating affected poultry farms and live bird markets. This marked the first occurrence of HPAI-H5N1 in the West African region since the last epidemic in 2006-2008. Since then, other countries in the region, namely Burkina Faso, Niger, Côte d'Ivoire and Ghana officially reported outbreaks of the disease. The scale and spread, and potential threat to human lives formed the basis of Member States' concerns and therefore an urgent request for assistance was made to both IAEA and FAO. APHL immediately reacted to the situation by taking advantage of the responsiveness of the FAO and IAEA programmatic structures. An emergency action plan was formulated to tackle H5N1 HPAI outbreaks in Western and Northern Africa. Thirteen Member States of the region were identified as targets, which included those that have already reported HPAI outbreak(s) and those bordering them with high risk for the spread of disease. As early and rapid diagnosis is the key for the control of this disease, emergency field support missions were organized with a "tool kit" of emergency

reagents and consumables to address the immediate diagnostic needs of national veterinary laboratories. This was followed by a refresher course to provide training on the early and rapid diagnosis of HPAI H5N1. Eleven Member States from the region were further provided with the required laboratory reagents and consumables to screen large numbers of suspected samples. APHL's response to this crisis was well appreciated both within IAEA and FAO as well as by several Member States, including but not limited to Côte d'Ivoire, Ghana, Burkina Faso and Togo.

The transboundary diseases affecting small ruminants continued to be the other major focus of APHL in 2015. Among them, peste des petits ruminants (PPR) was a primary focus with FAO and the World Organization for Animal Health (OIE) taking a lead in the development and implementation of a "Global Strategy for the Control and Eradication of PPR" beginning January 2015. In this regard, upon request from Member States, a real time PCR based multiparametric assay for the detection and surveillance of respiratory pathogens, including PPR, was transferred to eight national laboratories (Burkina Faso, Côte d'Ivoire, Democratic Republic of Congo, Ghana, Mali, Mongolia, Mozambique and Senegal). Further, a new diagnostic test for rapid detection of PPR in serum samples and for specific differentiation from antibodies against Rinderpest was developed and published by APHL. Full genome sequencing of PPR viruses also continued in order to better understand the molecular epidemiology of the disease.

In addition to PPR, the other important disease that causes significant economic losses to sheep and goat farmers worldwide is Capripox. During 2015, a molecular assay to differentiate sheep pox vaccine strains from field isolates of sheep pox virus (SPPV) and other Capripox genotypes was developed. With the increasing incidence of Capripox outbreaks in previously vaccinated herds, this test is expected to help in ruling out the involvement of vaccines in such outbreaks. Further, to improve the management of pox diseases in ruminants and camels, APHL developed a pan-pox real time PCR method and started transferring this assay to Member States.

With respect to African swine fever, the molecular epidemiological study was continued in 2015. Molecular characterization of ASFV in Côte d'Ivoire, Ethiopia, Mali, Mozambique, Nigeria and Tanzania was completed during this period. The results showed the continued presence of genotype I in Western Africa, genotypes I and IX in the DRC, and the presence of genotype II in Mozambique and Tanzania. Additionally, APHL initiated the development of a multiplex assay for the simultaneous detection and differentiation of African swine fever, classical swine fever, Salmonella and erysipelas in pigs. In the case of animal trypanosomosis, the effect of low dose irradiation on the expression of the parasite genome was evaluated using custom designed microarrays to identify genes that are responsible for the loss of virulence. Further, development of a dendritic cell based *in vitro* assay for cattle was initiated to evaluate immune response against irradiated trypanosome vaccine candidates.

Finally, under animal genetics, APHL completed genotyping large numbers of sheep and goats under field trials for testing the association with parasite resistant phenotypes. This was done as part of APHL's efforts to establish a low density marker panel for the selection and breeding of animals against haemonchosis, the parasitic disease that causes an economic loss of more than US $10 billion every year. A real time PCR based assay to discriminate three species of Haemonchus parasites was completed during 2015 and transferred to two Member States (Mozambique and Senegal) along with the required SOPs and reagent kit. Regarding the implementation of the Global Plan of Action for Animal Genetic Resources to protect livestock biodiversity, APHL supported Myanmar, Sri Lanka and Zambia on the molecular genetic characterization of indigenous buffalo, sheep and cattle, respectively. A meta-analysis was conducted to map molecular genetic diversity of indigenous goat breeds reared in nine Asian countries. Additionally, APHL staff in collaboration with staff at headquarters continued the development of a "Genetics Laboratory Information and Data Management System (GLIDMaS)". The development of various modules was completed and the system is ready for testing and further validation.

In addition to R&D, APHL was involved in capacity building activities in IAEA and FAO Member States. As part of those activities in 2015, APHL staff undertook seven technical field support missions (Côte d'Ivoire, Ghana, Mali, Mongolia, Mozambique, Niger and Senegal) to build capacity for animal disease diagnosis in national/central laboratories. APHL conducted three group training courses and hosted seven fellows and two interns, all funded by either extra-budgetary funds or by the IAEA Technical Cooperation (TC) Department, an indication of the strong linkage of APHL activities with those of TC.

APHL activities that were carried out in 2015, in particular capacity building activities, also benefited from the financial support of the IAEA Peaceful Uses Initiative (USA and Japan funded PUI projects) and the African Renaissance Fund (South Africa funded project).

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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Animal Health

Using irradiation technology to develop a potential trypanosome vaccine

Trypanosomosis, a parasitic disease in mammals, remains a big hindrance to the development of livestock resources in Africa. More than one third of Africa is infested by tsetse flies, the major insect vector of the parasite on the continent. The disease puts a large number of cattle at risk with annual losses estimated to be as high as US $5 billion. A vaccine would provide the most effective means of managing the disease in Africa and other endemic areas. At the Animal Production and Health Laboratory (APHL) of the FAO/IAEA Agriculture & Biotechnology Laboratories in Seibersdorf, experiments have been carried out to characterize the effects of low level irradiation doses on trypanosomes. Previous studies have shown that parasites subjected to such low doses are not able to cause an infection in mice. In addition, using low dose irradiated parasites induces a stronger immune response (with cytokine levels as a marker of immunity) when compared to using high dose irradiated parasites. In order to further study the effect of low dose irradiation on the expression of parasite genome, an expression micro-array platform that covers the genomes of three trypanosome species*, T. brucei, T. evansi* and *T. congolense*, has been designed by APHL.

Gene expression analysis of *T. evansi* parasites irradiated at varying doses ranging from 0Gy to 250Gy was conducted using the Affymetrix platform, with *T. brucei* as the reference sequence. At least six technical replicates per irradiation dose were used and initial results indicated that at least 3534 genes (*p>0.05*) were differentially expressed when subjected to different irradiation doses (Fig. 1). These results are now being analysed in depth to identify genes responsible for loss of virulence and infectivity. The classification of differentially expressed genes according to function (*i.e.* are the genes affected by irradiation responsible for structural, metabolic or enzymatic processes) will be important in deciding the targets for development of new vaccines and drugs. In addition, the differential expression pattern of irradiated *T. evansi* parasites will be compared to that of irradiated *T. congolense* to identify cross species targets.

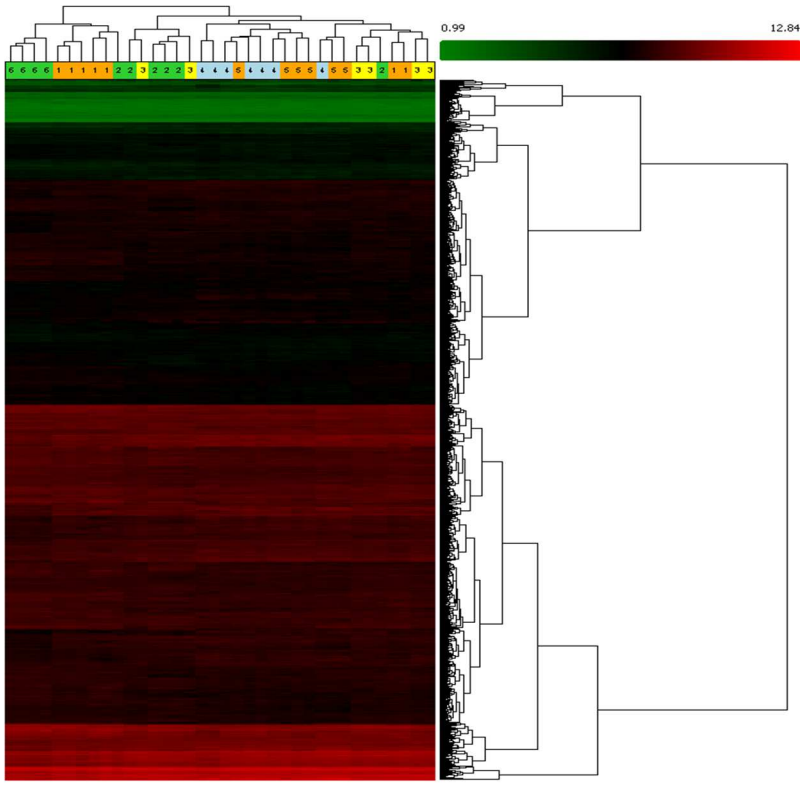

FIG. 1: Hierarchical clustering of differentially expressed genes in T. evansi *cultures subjected to different doses of irradiation ranging from 0Gy to 250Gy. Clusters in green represent repression in expression and those in red induction, both when compared to 0Gy*

Peste des petits ruminants

Peste des petits ruminants (PPR) is a highly contagious infectious viral disease of domestic and wild small ruminants. In its acute form, this disease is characterized by fever, depression, lack of appetite, respiratory distress, discharges in eyes and nose, diarrhoea and death in 80–100% of severe cases. After it was first identified in Côte d'Ivoire in 1942, it has spread to more than 70 countries in Asia, Africa and Middle East with an estimated annual loss ranging from US $1.45 billion to $2.1 billion. In 2015, the Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE) took a lead in developing and implementing a global strategy for the control and eradication of PPR. The APHL has been playing an active role in the development of tools for the control of this disease, in particular specific and rapid diagnostic tests and the generation of genetic data to better understand the molecular epidemiology of the disease and the evolution of PPR virus (PPRV), both important to prevent the transboundary spread of this economically important disease.

Molecular Epidemiology of PPR

Characterization of PPR virus from Benin

PPR is endemic in Benin, situated in West Africa, the second country after Côte d'Ivoire to report PPR. In the current study, PPRV was isolated from pathological and swab samples collected 42 years apart (1969 and 2011) in Benin and the full genome of two isolates (PPRV Benin/B1/1969 and PPRV Benin/10/2011) were sequenced. Phylogenetic analysis showed that all the characterized viruses clustered within the lineage II clade and that the 2011 isolates separated into two distinct subgroups. Comparison of the genomes revealed a 95.3% identity at the nucleotide level while at the protein level the matrix protein was the most conserved between the two viruses with an identity of 99.7% and only one amino acid change over the 42-year period. The V protein, with an identity of 93.1%, was the least conserved protein. An analysis of specific amino residues of known or putative function did not identify any significant changes between the two viruses. A molecular clock analysis of complete PPRV genomes revealed that the lineage II viruses sampled here arose in the early 1960's and that these viruses have likely persisted in Benin since that time.

Full genome sequencing of PPR virus

Four major lineages of PPRV have been reported to circulate among small ruminants across Asia and Africa. Full genome sequence information on different PPRV lineages helps in better understanding the evolution and spread of this important disease. During 2015, full genome sequences of PPRV from Liberia and Côte d'Ivoire were generated and submitted to public databases (e.g. GenBank). Both viruses have been shown to belong to lineage II and are therefore similar to PPRV circulating in the region.

New Serological Test (PPR-LIPS) for Surveillance of Peste des Petits Ruminants

PPR became the next target for control and eradication of transboundary animal diseases after the official declaration of global freedom from Rinderpest in 2011. Since both diseases are caused by closely related Morbilliviruses, the currently available tests for serological detection of PPR still present some antibody cross-reactivity. A new diagnostic test for rapid detection of PPR in serum samples and for specific differentiation from antibodies against Rinderpest was recently developed by APHL. The test, PPR-LIPS, is based on luminescence and, since it maintains the conformational structure of the proteins involved, is capable of differentiating PPR from related Rinderpest virus without cross-reactivity (Fig. 2). Final studies were carried out in 2015 using experimental and field sera samples and compared to Rinderpest to test for cross-reactivity. Additional comparisons were done using commercially available ELISA tests and viral neutralization tests. The PPR-LIPS showed high sensitivity and specificity and, unlike commercially available ELISA tests, showed no cross-reactivity to Rinderpest. Efforts are currently underway to transfer this new assay to Member States in 2016 for the sero-surveillance of PPR.

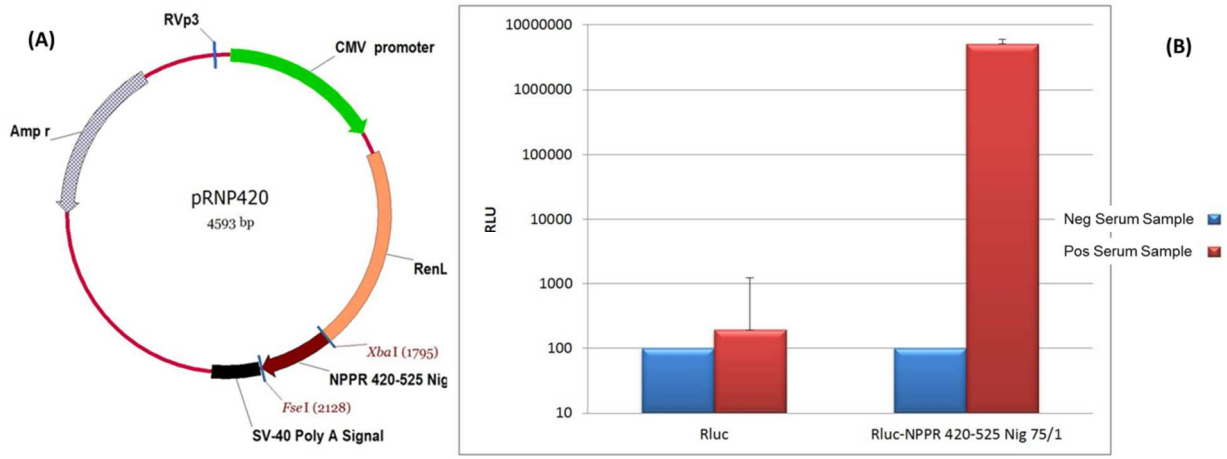

FIG. 2: (A) Structure of the pRNP420 mammalian expression vector. Some of the features indicated are the cytomegalovirus (CMV) promoter, the Renilla luciferase-NPPR 420-525 from Nigeria 75/1 fusion protein gene and the position of the two restrictions enzyme sites used for cloning. (B) Positive and negative PPR serum samples tested with PPR-LIPS. Negative serum (blue) from an Austrian goat; Positive serum (red) from PPR vaccinated sheep challenged with the India/Calcutta 95 strain

African swine fever

African swine fever (ASF) is one of the most devastating transboundary animal diseases for swine producers in Africa as well as in the Balkan and Caucasus regions. The disease also represents a serious threat to Europe with the recent detection of ASF in wild boar in several countries (including Russia, Belarus, Lithuania, Poland, and more recently Latvia and Estonia), showing the continued movement of virus within Europe. APHL continued to collaborate with Member States to assess the epidemiology of ASF virus (ASFV) and to study the viral genome.

During 2015, APHL assisted Member States in the detection of ASF within their borders. Real-time PCR technology was transferred to Ghana, Mali and Mozambique for ASF detection during suspected outbreaks. Also, the molecular characterization of ASFV in Côte d'Ivoire, Ethiopia, Mali, Mozambique, Nigeria and Tanzania, was completed during this period. Molecular characterization has shown the continued presence of genotype I in Western Africa, genotypes I and IX in the DRC, and the presence of genotype II in Mozambique and Tanzania. Phylogenetic analysis revealed the presence of a new genetic variant of ASFV in Ethiopia, which emphasizes the continued need to detect and characterize circulating ASFV strains to be able to provide information that could lead to the production of a successful vaccine.

Capripox disease

Capripoxviruses are responsible for economically important diseases of ruminants. Sheeppox virus (SPPV), goatpox virus (GTPV) and lumpy skin disease virus (LSDV), the three members of the genus Capripoxvirus (CaPV) of the *Poxviridae* family, affect sheep, goat and cattle, respectively. These three viruses are not strictly host specific and are antigenically very similar. Routine differentiation tools to allow accurate identification of pathogen is therefore essential to implement better control strategies for Capripox viruses.

Development of a Real Time PCR Method to Differentiate SPPV Vaccines from Field Isolates of Capripoxviruses

The main tool for Capripox control in disease endemic areas is vaccination. Live attenuated vaccines are preferred as they provide better and longer protection. However, using live attenuated vaccines present some challenges when the disease occurs in a previously vaccinated herd. In order to facilitate the epidemiological investigations of outbreaks occurring in vaccinated sheep and goat

herds, APHL in 2014 developed and validated a molecular assay to differentiate field isolates of SPPV from SPPV vaccines derived from the Romanian and the Yugoslavian RM65 strains. Although this assay could clearly differentiate SPPV vaccines from all other Capripoxviruses (CaPV), it was not possible to distinguish different CaPV genotypes (LSDV, GTPV and SPPV), thus requiring the use of an additional assay. In order to produce an assay that could discriminate SPPV vaccines from CaPV field isolates and further assign each of the CaPV field isolates into one of the CaPV genotypes (LSDV, GTPV or SPPV), the full genomes of several CaPV vaccine strains and field isolates from all genotypes were compared. Markers for differentiation were identified in the CaPV homolog of variola virus B22R. The B22R gene of

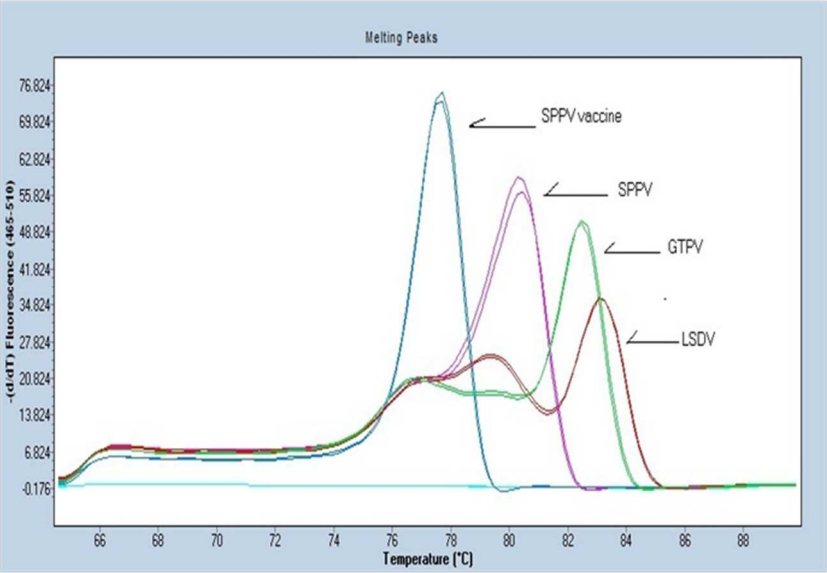

FIG. 3: Fluorescent melting curve analysis of PCR amplicons. Four different melting peaks, corresponding to SPPV vaccine, SPPV field isolate, GTPV and LSDV are displayed

SPPV vaccine contained stretches of deletions that were absent from all field isolates of SPPV, LSDV and GTPV. A region of this gene containing additional mutations for genotype discrimination was targeted to design a real time PCR assay based on high resolution melting (HRM) principle. The assay was optimized, evaluated and validated. The results showed that the SPPV vaccine could be distinctly differentiated from CaPV field isolates from all three genotypes (Fig. 3). Furthermore, the field isolates could be further segregated into one of the LSDV, GTPV or SPPV genotypes. The following melting peaks were observed: 77.62°C, 80.31°C, 82.45°C and 83.16°C for SPPV vaccine, SPPV, GTPV and LSDV, respectively. This assay uses only one primer pair and double stranded DNA intercalating dye thus making it cost effective. Furthermore the melting curves can be directly used for results interpretation, without the need for specialized HRM analysis software.

Expression and Evaluation of Recombinant Capripox Viral Antigens for Use in the Specific Detection of Capripoxvirus Antibodies

Capripox infections are currently expanding worldwide. Particularly, LDSV has recently demonstrated an unusual ability to spread and expand into new geographical areas in the Middle East and Europe. With the rapid movement of Capripox diseases, there is an urgent need for a high throughput technique for use in surveillance programmes to screen large numbers of animals in areas at risk. Such a tool will also serve for the screening of animals before export. Unfortunately, there is no commercially available ELISA for Capripox surveillance.

Current testing of truncated CaPV antigen has shown promising results (Fig. 4). Further studies on the validation of the assay and the determination of diagnostic performance are ongoing. This tool will facilitate surveillance in both endemic and disease free areas.

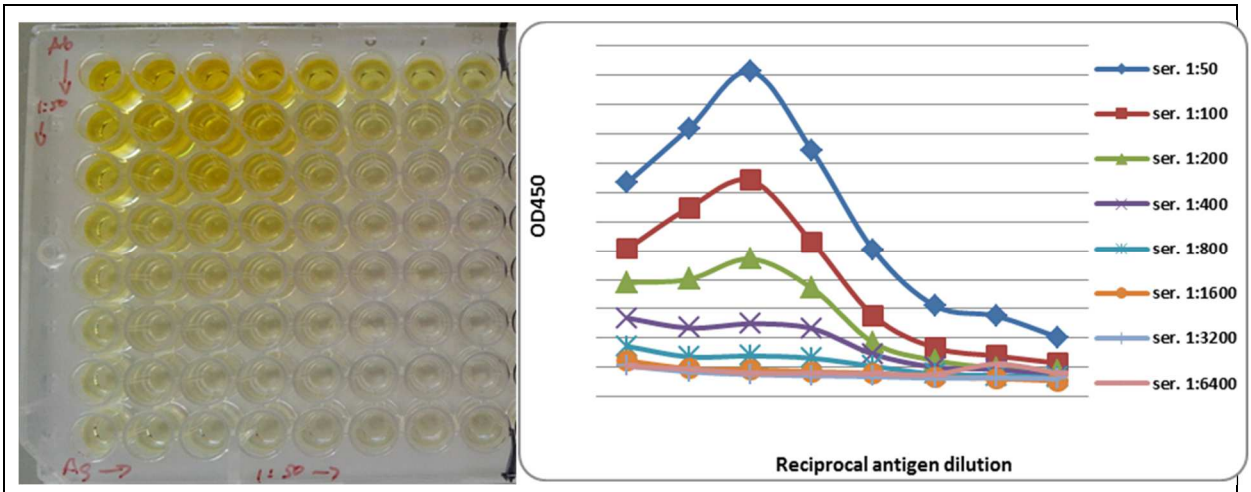

FIG. 4: Checkerboard titration of antigen and antibody where the process involved reciprocal serial dilution of the two reagents against each other.

Orf virus infections in sheep and goats in Ethiopia

Orf is an acute, contagious, debilitating and economically important zoonotic viral skin disease of sheep, goat and wild ruminants caused by orf virus (ORFV), a member of the Parapoxvirus genus. Owing to the existence of several diseases (such as sheep pox, goat pox, peste des petits ruminants, dermatophylosis and foot and mouth disease) that can potentially present similar lesions on the mouth and related symptoms (Fig. 6), laboratory diagnostics are needed for confirmation of Orf outbreaks.

With the view of developing a pan-poxvirus assay for detection of pathogens causing pox-like lesions in ruminants and cattle, APHL completed a comprehensive study of ORFV infections in sheep and goats in Ethiopia, between 2008 and 2013. Six orf outbreaks were investigated in different geographical locations of the country and the samples were taken to APHL for molecular characterization as well as for the validation of a pan-poxvirus assay. The results provided the first laboratory confirmation of ORFV infections where diagnosis was based only on clinical observations. Additionally, multiple variants of ORFV were characterized, highlighting at least two separate evolutionary pathways for ORFVs in Ethiopia. The current results will serve as a basis to formulate recommendations for ORFV management in the country. Additionally, these investigations have confirmed the need for a pan-poxvirus detection assay that can accurately identify pathogens causing pox-like lesions in ruminants and camels. Indeed, ORFV were originally characterized in samples suspected for Capripox infections.

Study of pox diseases in Ethiopian camels

Camels are economically important animals that are well adapted to arid and semi-arid climates and are valued for nomadic pastoralism, transportation, racing and production of milk, wool and meat. Two major pox diseases are known in camels: camelpox and camel contagious ecthyma. Camelpox is an infectious disease caused by camelpox virus (CMLV) of the genus Orthopoxvirus of the family Poxviridae. Camel contagious ecthyma, also known as Auzdyk disease (Fig. 5), is caused by camel contagious ecthyma virus (CCEV), a subclade of pseudocowpox virus (PCPV) in the genus Parapoxvirus of the Poxviridae family.

As part of its efforts to improve the management of pox diseases in ruminants and camels, APHL has developed a pan-pox real time PCR method and started transferring the assay to Member States. The current assay was used in Ethiopia as a front-line assay to investigate pox diseases in camels. This assay allowed the clear identification of CMLV and CCEV as two major causes of skin diseases of camels in the country. To further investigate and understand the epidemiology of these diseases, representative samples, collected from diseased camels (between 2011 and 2014) located in

different geographical regions of Ethiopia, were molecularly analysed. The full hemagglutinin gene for CMLV (HA, 948 bp) and major envelope protein gene (B2L, 1137 bp) for CCEV of Ethiopian isolates were amplified, sequenced and compared to publicly available sequences. The results confirmed the circulation of CMLV and CCEV among one-humped camels (*Camelus dromedarius*).

Twenty-seven CMLVs and 20 CCEVs were identified from pox disease outbreak samples collected in Ethiopia and compared to foreign isolates. Three major clusters of CCEV were found in Ethiopia, with cluster 1 isolates closely related, but not identical to Sudanese CCEV. The Ethiopian CCEV were closer to PCPVs retrieved from cattle worldwide than other parapoxviruses. For CMLV, all Ethiopian isolates formed a single cluster. They were closely related to CMLVs from Somalia and Syria. This study also highlighted the existence of CMLV and CCEV co-infections in two samples collected in suspicion of camelpox. This further highlights the challenges in clinically diagnosing CMLV infections due to the similarity of the clinical symptoms with CCEVs.

FIG. 5: Contagious ecthyma in young camels. Note the presence of severe nodular lesions on the upper and lower lips and around nostril

Real time PCR based multiplex assays for detection of pathogens

Two multi-parametric assays based on real time PCR developed at APHL in 2014 were further validated for the detection of respiratory pathogens in sheep and goats. The targeted pathogens include PPRV, CaPV, Orf virus, *Pasteurella multocida* and *Mycoplasma capricolum subspecies (ssp.) capripneumoniae (Mccp).* For the assay validation, three hundred pathological samples suspected of PPRV infections from Tanzania were screened for detection of respiratory pathogens. These samples were negative to PPRV, but a few samples were found positive for Orf virus indicating the advantage of the multiplex assays in disease surveillance and monitoring programmes.

The multi-parametric assay for the detection of respiratory pathogens in sheep and goats were transferred to Member State laboratories in Burkina Faso, Côte d'Ivoire, Democratic Republic of Congo, Ghana, Mali, Mongolia, Mozambique and Senegal, . The transfer was carried out through field support missions of APHL staff and fellowship training of scientists from these countries at APHL. Additionally, laboratory supplies and necessary equipment were provided to enable the use of these assays. The feedback obtained from countries show that the laboratories are now using the assays for surveillance and routine testing purposes. APHL provided additional support by assessing the initial run data and results from each laboratory and assisted in the interpretation of the results. It is expected that this assay will facilitate the necessary surveillance programmes required to implement the global strategy for the control and eradication of PPR. This strategy includes the control of other priority small ruminant diseases such as CCPP and Capripox, which are included in the panel of multiplex assays developed at APHL.

Development of in vitro *dendritic cell based assay to evaluate vaccine candidates*

The vaccine development process involves preclinical testing in experimental animals followed by clinical trials in the specific host. This is a lengthy, time consuming and expensive process (usually 10-15 years) involving significant resources. *In vitro* assays that simulate animal experiments can help in assessing vaccine candidates in a high throughput manner. Although animal experiments are the gold standard before the clinical trials, *in vitro* assays that simulate comprehensive host immune response may be an alternative. In this context, a dendritic cell (DC) based assay is being developed and evaluated at APHL.

In the initial experiments, this system was developed for cattle and the *in vitro* development of DCs from monocytes was successfully done. A DC-lymphocyte co-culture system was also developed and

intra cellular cytokine production (i.e. interferon gamma, IFN-g) from CD4 and CD8 T cells was measured as an immune response. Presently, this system is used to evaluate irradiated trypanosome vaccine candidates (Fig. 6). In the future, the system will also be optimized to carry out experiments to evaluate vaccine candidates for other species of animals and diseases.

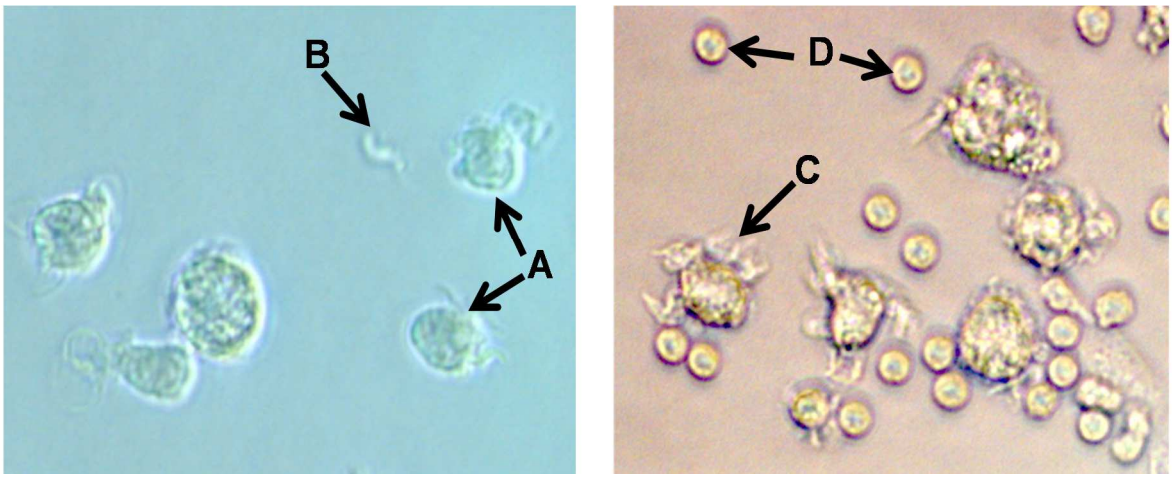

FIG. 6: In vitro *assay for irradiated trypanosome vaccine candidates. Bovine monocytes were cultured for 6 days with GM-CSF and IL-4 to induce immature DCs (A) and cultured overnight with irradiated (0-250Gy* Trypanosoma evansi *(B). Upon maturation, DCs (C) were harvested, washed and cultured with homologous lymphocytes (D) for another 7 days with pulsing crude lysate of* Trypanosoma evansi *on day 3 and 5 before measuring the production of IFN-g and cell proliferations.*

Animal Genetics

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

Gastro-intestinal (GI) parasitic infestations incur huge economic loss to poor and marginal farmers rearing sheep and goats across the world. The loss per year has been estimated at US $10 billion. Breeding programs with the goal of enhancing host resistance to parasites should help alleviate this problem in the long term.

Large Scale Genotyping of Sheep and Goat under Field Trial for Parasite Resistance

In continuation of its efforts to establish a low density DNA marker panel for parasite resistance, APHL developed genotyping assays for novel candidate gene SNP (single nucleotide polymorphism) markers to be associated with phenotypes. Large scale genotyping of 1367 goat samples derived from field trials performed in Bangladesh, China, India, Myanmar, Nigeria, Pakistan and Sri Lanka, were completed. A panel of 141 goat SNP markers located in 72 candidate genes, including pattern recognition receptor genes (toll like receptors, NOD like receptors, RIG I like receptors, C type lectin binding receptors), cytokine genes (e.g. interleukins, interferons) and caprine histocompatibility genes, was used to type 18 indigenous goat breeds for evaluation of parasite resistance. In the case of sheep, 1524 samples derived from field trials performed in Argentina, Brazil, Ethiopia, India, Indonesia and Iran were completed. A panel of 174 sheep SNP markers was used to type ten indigenous sheep breeds for evaluation of parasite resistance. Datasets of genotypes from a total of 3115 sheep and 1367 goats have been generated and statistical analysis is currently in progress in collaboration with counterparts from several Member States.

Genome Wide Association Study (GWAS) for Parasite Resistance in Sheep

In addition to candidate gene polymorphisms, genetic variations located throughout the genome play a significant role in the inheritance of traits related to parasite resistance. Hence, genome wide analysis of Corriedale sheep from Argentina, exhibiting extreme phenotypes, was initiated. Forty-eight sheep with low EBVs (estimated breeding values) for faecal egg count (supposed to be

relatively resistant/tolerant sheep) and 48 sheep with high EBVs for faecal egg count (supposed to be relatively susceptible sheep) were completed using a 60K Affymetrix microarray. Genotyping will be extended to additional sheep samples with phenotype extremes and to other breeds to perform a genome wide association study and identification of genomic regions under selection for parasite resistance.

Validation of a Real Time PCR Based Assay to Differentiate Sympatric Haemonchus Species Infecting Ruminants

Identification of different species/variants of *Haemonchus* parasites, as well as knowledge regarding the epidemiology and genetic characterization of the principal circulating species/variants, is essential for the establishment of sustainable control strategies. APHL initiated the development of a real time PCR (polymerase chain reaction) based assay to differentiate the three major *Haemonchus* species infecting ruminants in Asia. A novel snapback PCR assay coupled with melting curve analysis was developed in 2014. APHL continued the validation of this assay with additional field samples from Argentina, Austria and Nigeria. The specificity and sensitivity of the assay were also assessed to rule out cross reaction with other gastro-intestinal parasites and to establish the limit of detection, respectively. The results showed the assay to be highly sensitive and robust in detecting different species of *Haemonchus* (*H. contortus, H. placei* and *H. longistipes*) (Fig. 7). The assay works well with three different real time PCR platforms (BioRad-CFX, Roche-LightCycler and ABI-QuantStudio6) and is now ready for transfer to Member State laboratories. A reagent kit and an SOP have been developed and the assay was transferred to Mozambique and Senegal in 2015.

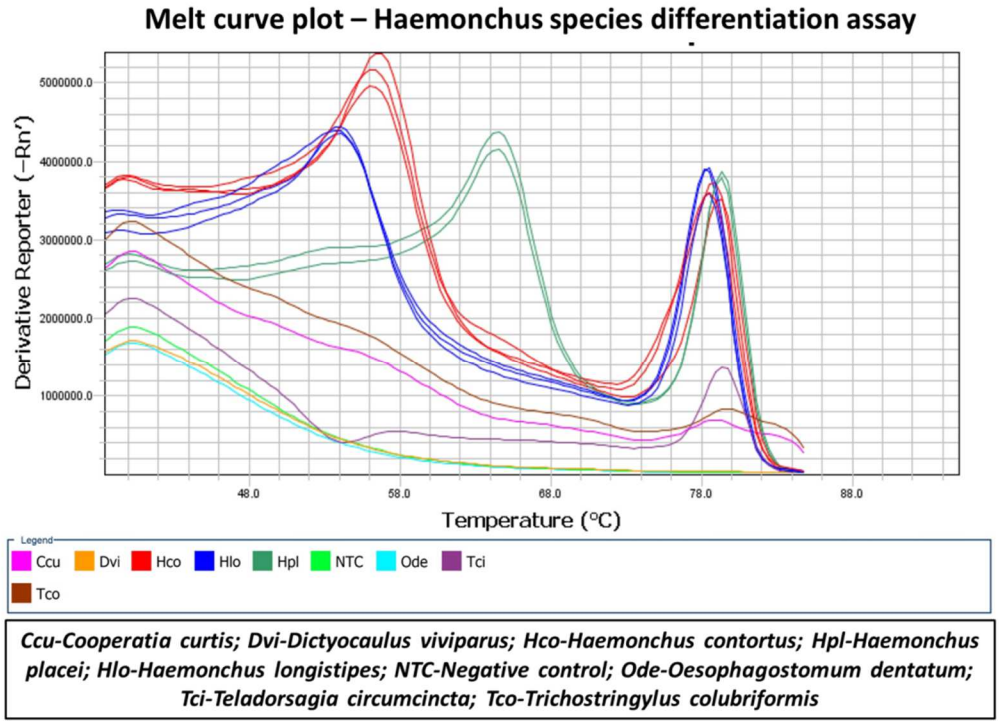

FIG. 7: Melting curve analysis of PCR amplicons showing specific melting peaks for each of the three sympatric Haemonchus *species. Absence of peaks for other related species (Ccu, Dvi, Ode, Tci, Tco) confirms the specificity of the assay*

Support to MSs for implementation of the Global Plan of Action for Animal Genetic Resources

The Joint FAO/IAEA Division is supporting Member States in implementing the Global Plan of Action for Animal Genetic Resources (AnGR) through capacity building and training. APHL supported

Myanmar, Sri Lanka and Zambia on molecular genetic characterization of indigenous buffalo, sheep and cattle, respectively, using nuclear and extra-nuclear DNA markers. Further, a meta-analysis was conducted to map molecular genetic diversity of indigenous goat breeds reared in nine Asian countries.

Mapping Molecular Diversity of Indigenous Goat Genetic Resources of Asia

The world goat population is approximately 1.0 billion with more than half of them present in Asia. The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture initiated a programme to characterize goat genetic resources of Asia. Nine Asian countries, namely Bangladesh, China, Myanmar, India, Indonesia, Iran, Pakistan, Sri Lanka and Vietnam, were supported to conduct breed surveys, evaluate production environments and assess phenotypic and genetic characteristics of indigenous breeds/populations. A meta-analysis of genotypes from 2249 goats belonging to 57 goat breeds located in these countries was conducted to assess genetic diversity, relationship and population structure. Genetic differentiation among goat breeds/populations within countries varied from 1.9% (Myanmar) to 12.6% (Indonesia) with a global F_{ST} of 12.7%. Genetic differentiation among local goats within countries was limited, an indication of high gene flow across breeds/populations. The microsatellite based phylogeny showed two major clades: the Chinese goats clustered distinctly while the goat breeds from other countries clustered separately into a single clade. Weak genetic structure was observed in Bangladeshi, Sri Lankan and Myanmar goats, moderately strong genetic structure was observed in Pakistani goats while strong genetic structure was observed in Indonesian, Iranian, Vietnamese and Chinese goats. Model based cluster analysis of the metadata broadly grouped Asian goats into two major geographical clusters (Chinese and West Asian), which can be further partitioned into four groups: Chinese, West Asian, South East Asian and South Asian. The results of the meta-analysis clearly established the genetic distinctness of Chinese goats from other major Asian goat breeds.

Genetic Characterization of Indigenous Sheep of Sri Lanka

Small ruminants (sheep and goats) form an important component of the livestock production system in Sri Lanka. Sheep and goats are mostly reared for meat and crossbreeding has been applied to improve the production performance of local animals. This resulted in genetic dilution of indigenous breeds/populations, though little or no information on their genetic characteristics is available. The Joint FAO/IAEA Division supported Sri Lanka's effort on genetic characterization of indigenous goat populations of Sri Lanka through a coordinated research project. As an integral part of this, APHL supported the genetic evaluation of Jaffna Local sheep, the only indigenous sheep breed of Sri Lanka. DNA samples collected from various flocks of Jaffna Local sheep were genotyped using short tandem repeat DNA markers, single nucleotide polymorphic markers and mitochondrial DNA markers. Genotype and sequence data was compared with south Indian sheep to assess the genetic relationship, particularly of Madras Red, the sheep breed recommended for crossbreeding to improve mutton production. Information generated by this work is expected to help in formulating strategies for breeding, improvement and conservation of indigenous sheep in Sri Lanka.

Genetic Characterization of Indigenous Buffalo Populations of Myanmar

Water buffalo (*Bubalus bubalis*) is an important livestock in south and southeast Asia. Buffaloes are valuable not only as milk producers, but have multiple roles in rural livelihoods, particularly as a draught animal in paddy cultivating areas where they contribute considerably to employment generation and nutritional security. Myanmar has raised buffaloes for draught purposes since time immemorial, with an estimated population of 2.6 million. Although most buffaloes are considered to be swamp type, river buffaloes are also available in some areas. Characterization and documentation of these buffalo populations is of prime significance for genetic improvement and biodiversity conservation programmes in Myanmar. APHL initiated and completed the design, development and optimization of DNA marker panels for genetic characterization of water buffaloes. Six multiplex panels covering 21 microsatellite DNA markers were standardized for genotyping; the

marker panels performed well for characterization of both sub-species of buffaloes, river and swamp types. Indigenous buffalo populations from three different provinces of Myanmar were genotyped and sequenced (mitochondrial DNA D-loop variations). Statistical analysis of genotype and sequence data is currently in progress.

Genetics Laboratory Information and Data Management System

APHL continued its efforts to develop a Genetics Laboratory Information and Data Management system (GLIDMaS) to support FAO/IAEA Member States in managing their livestock biodiversity and improving productivity of local animal breeds. The GLIDMaS platform will be a standalone application and will need no special software on user computers. The system will have the facility to manually enter and edit data, import multiple datasets from spreadsheet, search different modules, export searches and found items and create reports (Fig. 8). Development of all the modules (genetic repository, DNA marker-microsatellites, DNA marker-SNP, DNA sequence, RH panels, oligos and SOPs-protocols-manuals) have been completed. Validation of manual data entry and bulk data import is currently in progress.

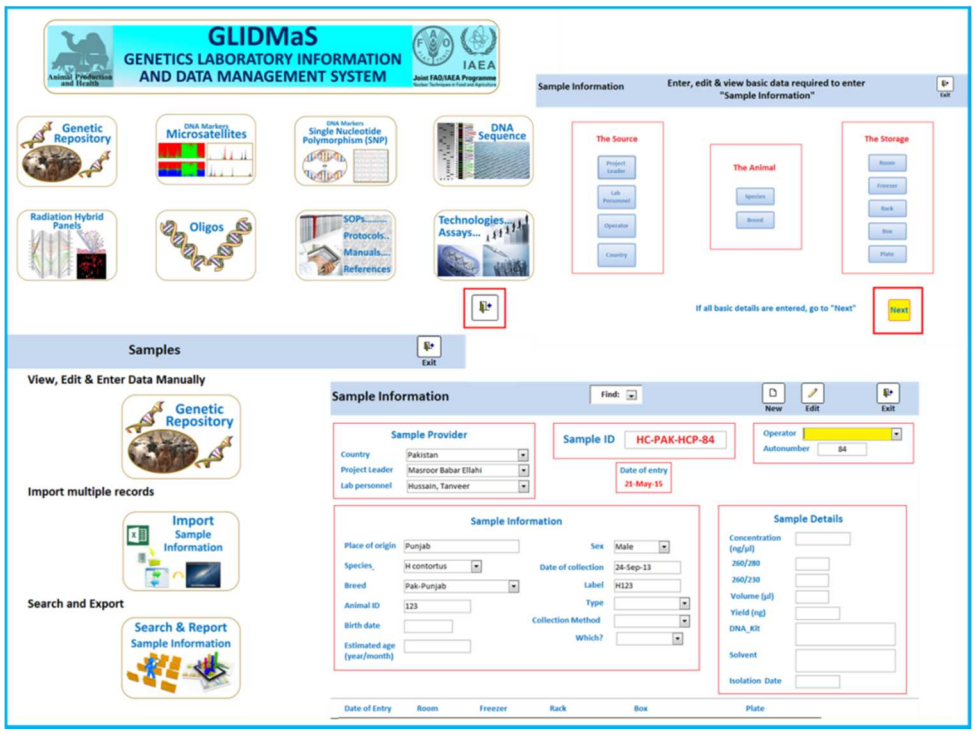

FIG. 8: The Genetics Laboratory Information and Data Management System

CAPACITY BUILDING

Emergency response to enhance Member State diagnostic capacities for tackling the H5N1 avian influenza crisis in western and northern Africa

Early and rapid diagnosis is a key to halt the spread of avian influenza and to control the disease at its outbreak site. This requires direct support and guidance to national veterinary laboratories that need to be prepared to meet this challenge. Upon requests from Member States in Africa, an emergency action plan was formulated to tackle H5N1 highly pathogenic avian influenza (HPAI-H5N1) outbreaks in western and northern Africa. Thirteen Member States (Benin, Burkina, Burundi, Cameroon, Central African Republic, Chad, Côte d'Ivoire, Ghana, Libya, Mali, Niger, Senegal and Togo) were identified to be targets for the implementation of the action plan. This included countries that have already reported HPAI outbreak(s) and those bordering them with high risk for the spread of disease. APHL utilized its VETLAB and the OIE-FAO OFFLU networks to reach out to

national and central veterinary laboratories in these countries. Technical requirements of these laboratories in terms of (a) technologies for diagnosis and typing of causative viruses, (b) laboratory equipment and supplies to analyse suspected samples from the field and (c) capacity of laboratory personnel in performing the diagnostic and typing assays were identified. Based on the information collected, an emergency action plan was implemented in May 2015 under the joint leadership of IAEA and FAO.

a. **Field support missions:** As a first step of the emergency action plan in enhancing Member State capacities, field support missions were undertaken by APHL staff with a "tool kit" of emergency reagents and consumables to address the immediate diagnostic needs of national veterinary laboratories in Côte d'Ivoire, Ghana, Mali, Niger and Senegal. . All laboratories initially had insufficient technical knowledge on the diagnosis of H5N1-HPAI. APHL staff also installed diagnostic equipment in three of the laboratories (Côte d'Ivoire, Ghana, Mali) and conducted on-site training of laboratory personnel in the handling suspected samples (adhering to biological safety standards), diagnostic and typing assays and interpretation of results. The missions proved very successful in the rapid diagnosis and notification obligations to OIE and resulted in effective, fully equipped and functional facilities for HPAI-H5N1 diagnosis in each of these countries.
b. **Provision of reagents and laboratory supplies:** To address the gap of deficiencies in laboratory reagents and supplies to handle large numbers of suspected samples, diagnostic toolkits, reagents and consumables, along with guidance and standard operating procedures for HPAI-H5N1 diagnosis, were provided to eleven countries in western and northern Africa (Burkina, Cameroon, Chad, Côte d'Ivoire, Ghana, Libya, Mali, Niger, Senegal, Togo and Benin). This will help these countries to implement effective surveillance measures and monitor HPAI for several months.
c. **Refresher training courses for emergency preparedness to tackle HPAI outbreaks:** In parallel to the field support missions and the provision of laboratory supplies, a refresher training course was organized in September 2015 on the early and rapid diagnosis of HPAI-H5N1. Twelve participants from ten Member States participated (Benin, Burkina Faso, Burundi, Cameroon, Central African Republic, Côte d'Ivoire, Ghana, Niger, Togo and Zimbabwe). At the end of the course, each participant returned to their respective laboratories with an emergency toolbox containing all the reagents necessary to perform the immediate screening for HPAI-H5N1 in more than 200 samples.

Technical field support missions to build capacity in MS veterinary diagnostic laboratories

APHL staff was actively involved in the transfer of technologies to IAEA Member States and during 2015 seven technical field support missions were undertaken to install and calibrate critical equipment and to demonstrate work flow on disease diagnostic procedures, surveillance and epidemiology of transboundary animal diseases. Several of these missions also focused on building national capacities in the diagnosis of H5N1 avian influenza.

Laboratoire Central Veterinaire de Bingerville, Abidjan, Côte d'Ivoire

APHL staff travelled to Abidjan, Côte d'Ivoire from 25-29 May, 2015 to transfer animal pathogen typing technologies to the Laboratoire Central Vétérinaire de Bingerville. The aims of this mission were (1) to respond to the emergency H5N1 situation in Côte d'Ivoire, (2) to provide an emergency "tool kit" and (3) to transfer and implement animal disease diagnostic technologies and build capacities. During the week, the laboratory was assisted in setting up instruments, and staff were trained on animal pathogen detection by real time nucleic acid amplification technology, including multi parametric pathogen detection assays. To date, the laboratory has provided valuable feedback on the successful adoption of the rapid detection and subtyping of H5N1 using a duplex real time PCR assay. The assay is now used as a front-line tool by the laboratory for the screening of suspected H5N1 cases in support of national effort to control the HPAI crisis in the country. Moreover, the

laboratory was also able to use sequencing technology for full characterization of the samples from one outbreak. This achievement highlights the dynamism of the laboratory and the importance of the capacity building approach adopted by APHL.

Laboratoire Central de l'élevage, Niamey, Niger

In January 2015, Nigeria confirmed the presence of HPAI-H5N1 to the OIE while neighbouring Burkina Faso and Niger reported outbreaks in April 2015. A field support mission was made to Laboratoire central de l'élevage (LABOCEL), Niamey, Niger along with the provision of the above emergency "tool kit". During the four-day mission, the personnel were trained on a number of protocols through demonstrations and practical sessions. These included the isolation and purification of RNA from swab samples, conventional RT-PCR identifying the H5 gene in clinical samples, conventional RT-PCR identifying the N1 gene in clinical samples and a duplex real time PCR that simultaneously identifies the presence of the N1 and H5 genes in clinical samples. At the end of the mission, LABOCEL was able to implement all the assays for rapid diagnosis of H5N1 and acquired the capacity to screen large number of suspected samples.

Accra Veterinary Laboratory, Veterinary Services Directorate, Ministry of Food and Agriculture (MOFA), Accra, Ghana

An expert from APHL undertook this travel to assist Ghana in the rapid identification of H5N1 cases occurring in Ghana and potentially spreading from neighbouring countries such as Burkina Faso, Côte d'Ivoire and Nigeria. The objective was to introduce real time PCR technology for the diagnosis of HPAI-H5N1. Follow up correspondence with the laboratory director, Mr Joseph Awuni, has shown the continued use of this technology in rapidly and accurately detecting H5N1 in outbreak samples from Ghana, with data reports being sent on a regular basis to the APHL.

The Central Veterinary Laboratory, Maputo, Mozambique

This mission was undertaken in October 2015 by an expert from APHL, as part of a project to strengthen animal disease diagnostic capacities in selected Sub-Saharan African countries, supported by the South African Renaissance Fund (ARF). The mission included the transport and installation of a new real time PCR platform (CFX96 Bio-Rad). A number of molecular diagnostic protocols were successfully demonstrated on the instrument for the detection of important transboundary animal diseases, such as African swine fever, avian influenza and peste des petits ruminants. The laboratory staff was trained on the protocol for the identification and pathotyping of Newcastle disease virus (NDV).

Laboratoire National d'Elevage et de Recherches Vétérinaires, Dakar, Senegal

The main goals of this mission were to transfer new technology and coordinate future collaboration between the laboratory staff and the Joint FAO/IAEA Division regarding African swine fever. This laboratory has been very capable of performing real-time PCR assays, so the focus was to transfer the working knowledge of several new multiplex assays that have been developed at APHL. These real-time assays are based on multiple pathogen detection, whereby one animal sample can be tested for many pathogens in a single procedure. The first assay involved detection of *Pasteurella*, *Mycoplasma capricolum subsp. capripneumoniae* (MCCP), peste des petitis ruminants virus (PPRV) and Capripoxvirus from sheep and goats. Another assay was a pan-poxvirus detection procedure that included testing of eight pathogens that can be present with common clinical signs. This assay can between CPXV, CMPV, SPPV, GTPV, LSDV, ORFV, PCPV, and BPSV. Transfer was also done with the duplex HPAI-H5N1 real-time RT-PCR assay for avian influenza. While Senegal is currently not affected by HPAI, the mission helped to build capacity and prepare the laboratory for early and rapid diagnosis in case of cross border movement of the disease.

Laboratoire Central Veterinaire, Bamako Mali

APHL staff travelled from 31 August to 4 September to transfer animal pathogen typing technologies to the Laboratoire Central Veterinaire, Bamako, Mali. The mission was carried out under the framework of the project to strengthen animal disease diagnostic capacities in selected Sub-Saharan African countries, supported by the South African Renaissance Fund (ARF), and Peaceful Uses Initiative (PUI) projects supported by USA and Japan. The laboratory recently received the molecular diagnostic platform under the project and the mission was undertaken to transfer real time PCR technology, focusing on multi-targets detection, as an additional tool for a more rapid and accurate diagnosis of transboundary animal diseases. The laboratory staff was trained on well-established protocols, including those developed at APHL, as well as protocol selection procedures to facilitate the implementation of new assays. Fifteen scientists and technicians of all departments participated in the training.

The objectives of the training were successfully achieved and the laboratory staff was able to set up, execute and interpret the results of real time PCR assays for the detection of African swine fever, peste des petits ruminants viruses and CaPV genotyping. They were also able to perform and analyse the results of multi-parametric assays to detect pathogens responsible for respiratory diseases and pox-like lesions in ruminants. Given the recent emergency due to HPAI-H5N1 outbreaks in several west African countries, the mission also ensured adequate capacity for H5N1 diagnosis. An emergency "tool kit" of supplies for H5N1 diagnosis was provided along with the required training. This technology will help the laboratory to better fulfil its mandate within the national strategies for the control of transboundary animal diseases.

A scientist of the Laboratoire Central Veterinaire, Bamako, Mali, operating the real time PCR instrument

State Central Veterinary Laboratory, Ulaanbaatar, Mongolia

Following a request from Mongolia during the first technical meeting of Asian veterinary laboratories held at Vienna, a field support mission was conducted by APHL staff from 1-5 December 2015. The major focus of the mission was to transfer real time PCR based multi-parametric detection of pathogens causing respiratory diseases in small ruminants, red diseases in pigs (African swine fever, classical swine fever, Salmonella and Erysipelas) and pox like lesions in ruminants and camels. Six staff was given hands-on practical training on these techniques along with sequence data analysis. The team expressed satisfaction and confidence in running the multiplex assays, data interpretation and sequencing data analysis. The acquired knowledge will improve the laboratory's contributions to Mongolia's efforts in controlling transboundary animal diseases (TADs) affecting sheep, goat, pigs and camels.

Meetings

During 2015, two technical meetings were held at the IAEA's Headquarters in Vienna, Austria with directors of veterinary laboratories participating in the Peaceful Uses Initiative (PUI) and the African Renaissance Fund (ARF) projects to strengthen animal disease diagnostic capacities in, respectively, selected Asian and Sub-Saharan African countries.

Technical meeting with directors of Asian veterinary laboratories participating in the project to build and improve animal disease diagnostic capacities of veterinary laboratories in Asia

The first technical meeting was held from 23-25 March 2015 with representatives from five partner laboratories in Bangladesh, Lao People's Democratic Republic, Mongolia, Myanmar and Nepal. Based on the presentations from each of the partner laboratories, it was clear that the main TADs of interest included foot-and-mouth disease (FMD), HPAI, Newcastle disease, PPR and other small ruminant diseases such as Capripox, Parapox, Pasteurella and Bluetongue. Most of the laboratories possess a minimum capacity required to diagnose FMD, HPAI and NDV, although they needed to strengthen their capacity for the typing and characterization of HPAI and FMD (including vaccine matching). In contrast, little or no capacity is present in the participant countries for the diagnosis and characterization of PPR and other small ruminant diseases.

All countries highlighted the need to perform more investigations on small ruminant diseases. More specifically, PPR is of high importance to all of these countries and they need to implement an active surveillance of small ruminant respiratory diseases. As a result, it was agreed that a multiplex PCR developed by APHL for pathogens causing respiratory diseases in small ruminants, including PPR, will be transferred to all participant laboratories within the first year of the project. Additionally, it was agreed to strengthen capacity for pathogen detection by promoting the introduction of quality systems in individual laboratories. This includes the support for the implementation of a Laboratory Information Management System (LIMS), continuous support to provide external quality assessment (EQA), quality audit expert visits and the organization of equipment calibration and basic maintenance training. The introduction of rapid field diagnosis to support early pathogen detection in remote areas will also be addressed.

Technical meeting with Directors of veterinary laboratories participating in the project to strengthen animal disease diagnostic capacities in selected sub-Saharan countries

The second technical meeting with directors of African veterinary laboratories supported by the ARF and the PUI to strengthen animal disease diagnostic capacities was held at the IAEA Headquarters in Vienna, Austria, from 16-18 June 2015. Thirteen partner laboratories from Botswana, Burkina Faso, Cameroon, Chad, Democratic Republic of Congo, Ethiopia (2), Mali, Mozambique, Namibia, Senegal, Tanzania and Zambia participated. All participants provided updates on their progress and achievements in implementing the 2014 work plan, and on new emerging challenges. Two major challenges were the re-emergence of HPAI in west Africa and the high threat of peste des petits ruminants in southern African countries. Significant achievements since the first technical meeting included:

1. Accreditation of the National Veterinary Institute laboratory in Ethiopia;
2. Diagnostic services were provided by Laboratoire National Vétérinaire (LANAVET), Cameroon, to Chad on African swine fever, and to Gabon for several TADs.
3. The management of the recent HPAI outbreak in Burkina Faso by the Senegal Laboratoire National d'Elevage.
4. Increase in the number of assays under accreditation by the National Veterinary Laboratory in Botswana and the National Animal Health Diagnostic and Investigation Centre (NAHDIC) in Ethiopia.

The participants highlighted the significant contributions of this project, particularly in bringing together several laboratories of Africa and sharing their experience and knowledge. For instance, Burkina Faso shared their experience and challenges on HPAI crisis management at both laboratory and field levels, which was useful for countries not yet affected. Similarly, NAHDIC shared their experience in setting up a cost-effective biosafety level 3 laboratory that could be affordable also for many other laboratories in the network.

The current proficiency testing organized by APHL was strongly appreciated and it was agreed to extend this to Rift valley fever and CBPP detection.

Training courses

A training course on the "Early Detection of Animal Diseases in Post Flooding Environment, with Emphasis on Water Borne and Vector Borne Diseases" was held from 15-26 June 2015 at the IAEA Laboratories in Seibersdorf. Twenty three participants from 13 Member States of the Asian region (Bangladesh, Cambodia, China, Indonesia, Lao PDR., Malaysia, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Thailand and Vietnam) participated in the course. Four international experts covered the topics of the course: i) Leptospirosis; ii) Bluetongue and West Nile fever; iii) Clostridial infections of animals and iv) Disease mapping and modelling using geo information/visualization tools (GIS).

A training course on 'Transboundary Animal Disease Diagnoses: Sequencing and Bioinformatic Analyses of Animal Pathogen Genomes' was held from 9-20 November 2015 at the IAEA Laboratories in Seibersdorf. Seventeen VETLAB Network veterinary diagnostic laboratory scientists from 12 Sub-Saharan African and 4 Asian countries (Botswana, Cameroon, Chad, Democratic Republic of Congo, Ethiopia, Kenya, LAO PDR, Mali, Mongolia, Mozambique, Myanmar, Namibia, Nepal, Senegal, United Republic of Tanzania and Zambia) participated. The objectives of this training course were to promote the use of 'gene based identification and classification of pathogens' by veterinary diagnostic and research laboratories in Africa and Asia, and to strengthen the capacity of participant countries in genomic sequence analysis of pathogens causing zoonotic and transboundary animal diseases. Course participants were also informed of options for outsourcing their sequencing work to service providers and critically assessing the quality of the raw data received from these service providers. The theoretical and practical training was delivered by experts from the Swiss Institute of Bioinformatics, Lausanne, Switzerland, the Veterinary and Agrochemical Research Centre, Brussels, Belgium, the Friedrich Loeffler Institute, Greifswald, Germany and the Joint FAO/IAEA Division.

Fellowship and internship training

In 2015, the APHL hosted seven fellows and two interns in the following areas:

Name	Country	Status	Duration	Topic
Mapaco, Lourenço Paulo	Mozambique	Fellow	3 months	Laboratory diagnosis of transboundary animal diseases using molecular techniques
Kayesa, Edgar	Zambia	Fellow	3 months	Laboratory diagnosis of transboundary animal diseases using molecular techniques
Mangate, Keitumetse Gladys	Botswana	Fellow	4 months	Laboratory diagnosis of transboundary animal diseases using molecular techniques
Ngassa, Charles Mayenga	Tanzania	Fellow	3 months	Laboratory diagnosis of transboundary animal diseases using molecular techniques
Kyaw, Lwin Ko Ko	Myanmar	Fellow	3 months	Genetic characterization of indigenous buffaloes of Myanmar

Name	Country	Status	Duration	Topic
Chileshe, Brenda	Zambia	Fellow	3 months	Analysis of data for genetic characterization of Zambian native Zebu cattle using nuclear and extra-nuclear DNA markers
Sanou, Moumouni	Burkina Faso	Fellow	1 month	Genotyping Djallonke sheep for parasite resistance
Kurukulasuriya, Maheshika	Sri Lanka	Intern	3 months	Genetic diversity analysis of Sri Lankan sheep using nuclear and extra-nuclear DNA markers
Silbermayr, Katja	Austria	Intern	1 month	A novel real time PCR based snapback assay to differentiate sympatric species of *Haemochus*

PUBLICATIONS

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EXTERNAL COLLABORATIONS AND PARTNERSHIPS

Institution	Topic
Centre de Coopération Internationale pour la Recherche Agronomique et le Développement (CIRAD), France	PPR and capripox research
The Pirbright Institute, UK	Capripox research
National Animal Health Diagnostic and Investigation Center (NAHDIC), Ethiopia	Capripox research
National Veterinary Institute (NVI), Ethiopia	Capripox research
Pan African Veterinary Vaccine Centre (PANVAC), Ethiopia	Livestock vaccine quality
Laboratoire Central Vétérinaire (LCV), Mali	Capripox and PPR research
Laboratoire Vétérinaire de Kinshasa, DRC	ASF, PPR research
Institute for Veterinary Disease Control, Austrian Agency for Health and Food Security (AGES), Mödling, Austria	Exotic animal diseases research (Capripox, PPR, ASF)
Laboratoire National Vétérinaire (LANAVET), Cameroon	ASF
Special Pathogens Unit of the National Institute for Communicable Diseases, South Africa	RVF
Laboratoire National d'Elevage et de Recherches Vétérinaires (LNERV/ISRA), Senegal	Capripox, PPR, ASF
Tanzania Veterinary Laboratory Agency, Tanzania	ASF, PPR and Orf disease
OIE reference laboratory for ASF, Universidad Complutense de Madrid, Spain	ASF
Livestock Breeding and Veterinary Department (LBVD), Myanmar	Animal Genetic Resources
University of Peradeniya, Peradeniya Sri Lanka	Animal Genetic Resources and Haemonchosis
National Institute for Scientific and Industrial Research (NISIR), Zambia	Animal Genetic Resources
Universita Cattolica del Sacro Cuore,(UNICAT), Italy	Livestock biodiversity research
Department of Population Genetics, Veterinary Medical University (VETMEDUNI), Austria	Animal genetics and Haemonchosis

Institution	Topic
Département Productions Animales, Institut de l'Environnement et de Recherches Agricoles (INERA), Ouagadougou, Burkina Faso	Genetic improvement for parasite resistance
International Livestock Research Institute, Nairobi, Kenya	Animal Genetic Resources
Instituto Nacional de Tecnología Agropecuaria, Instituto de Genética, Buenos Aires, Argentina	Genetic improvement for parasite resistance
University of Forestry, Sofia, Bulgaria	Animal Genetic Resources and DNA bar coding
Friedrich-Loeffler-Institut, Greifswald - Insel Riems, Germany	Genetic markers for Scrapie in goats
Swiss Institute of Bioinformatics, Switzerland	E-learning and face-to face training

EXTRA-BUDGETARY SUPPORT

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

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

THE FOOD AND ENVIRONMENTAL PROTECTION LABORATORY

EXECUTIVE SUMMARY

The Food and Environmental Protection Laboratory (FEPL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture provides assistance to Member States in implementing food control systems to ensure the safety and quality of the food supply, safeguarding consumer health and helping to facilitate international trade. Technical support is provided for food provenance determination, contaminant control systems and for authenticity testing. This support underpins food safety and traceability systems and combats economic loss through the illegal production and marketing of counterfeit and adulterated products. Activities include applied research, the development, validation, transfer and application of nuclear and related methods such as stable isotope measurements and metabolomics for food authentication, isotope dilution assays for chemical contaminant detection and control, and radiotracer techniques to study contaminant transfer. The application of these technologies and methods in Member States is supported by the development and provision of technical protocols, advice and guidance, and inputs for the development of international standards.

Research and development achievements in 2015 included the development and evaluation of analytical methods to underpin food traceability systems and for food authentication, with a focus on important commodities in international trade and targets for fraudulent practices such as counterfeiting or adulteration. Methodology previously investigated in the FEPL employing metabolomics to facilitate the authentication of honey was further refined and applied to the differentiation of manuka and kanuka honeys from New Zealand – two economically important products that are extremely difficult to differentiate. A targeted method previously developed for the detection of adulteration of fruit juices was improved through the identification of additional biomarkers using untargeted metabolomics, thereby enhancing the robustness and applicability of the method. The method was tested for the authentication of Indian fruits and fruit juices in collaboration with research partners in India. A method for targeting only non-exchangeable hydrogen atoms in pectin extracted from foods was adapted to allow the measurement of stable hydrogen isotope ratios that provide information on the food's geographical origin. The method was tested using tea samples. The FEPL continued work as a research partner in the EU 7th Framework integrated project 'FoodIntegrity', and helped develop a new project proposal, 'Authent-Net', which was successful in gaining EU funding. The FEPL continued to coordinate and provide technical input to two coordinated research projects on food traceability and authenticity, involving thirty countries.

Achievements related to the control of residues and contaminants in food included the development and validation of a multi-residue method for pesticides in potatoes by gas chromatography - triple quadrupole mass spectrometry, as part of a larger study performed in the Red Analitica de Latinoamérica y el Caribe (RALACA) laboratory network. A method was also developed in collaboration with scientists from Uruguay for pesticides in the medicinal herb, boldo. The transfer of pesticide residues from boldo into the herbal tea made from the plant was characterised to provide risk assessment data. The FEPL participates in the CRP 'Development and Strengthening of Radio-Analytical and Complementary Techniques to Control Residues of Veterinary Drugs and Related Chemicals in Aquaculture Products', which commenced in 2015. This project includes 15 laboratories in 14 countries, plus the FEPL.

The results of FEPL research were presented at six international conferences, and the FEPL was represented in the scientific committees for two major international conferences on food safety and regulatory analysis for residues/contaminant control in food.

Capacity building activities in 2015 included the technical management of twenty national and five regional Technical Cooperation Projects. Three training workshops were organised with extrabudgetary funding by FEPL and held in Member States. More than one hundred and sixty

scientists, analytical chemists, laboratory personnel and food inspectors from more than fifty countries were trained through these workshops. The FEPL hosted six interns and three visiting scientists. The sustainable, formal network of food safety laboratories in Latin America and the Caribbean, RALACA, which was initiated and established with FEPL assistance in 2012, was expanded from sixteen laboratories to more than fifty laboratories and institutions in twenty one countries, with four new member institutes in 2015. RALACA is effectively promoting and supporting food safety and environmental sustainability in the region. The FEPL was represented in the Global Food Safety Partnership, providing input to the food safety technical working group and the laboratory capacity working group.

Publications by FEPL staff in 2014 included five papers in peer-reviewed scientific journals and five papers in conference proceedings/books of abstracts.

STAFF

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Pavkovic, Anita	Team Assistant

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Food traceability and authenticity

Verification of origin is an essential component of a food control system. Traceability refers to the ability to follow the movement of a food through specified stage(s) of production, processing and distribution. Origin refers to the point where produce was harvested or where an animal was reared.

The ability to independently verify food origin provides a necessary audit of the traceability. There are numerous examples of the use of chemical analysis of food to determine its origin. These techniques rely on defining a specification for authentic food from measurements of a reference sample set. Data from an unknown or test sample can then be compared with these authentic sample data sets to confirm authenticity. This approach can be very robust but tends to be slow and expensive to implement. Research at FEPL aims to make such verification technology more accessible to Member States through development or adaptation of nuclear techniques for reliable, faster and cheaper screening and response.

Differentiation of manuka and kanuka honeys by mass spectrometry and chemometrics

New Zealand manuka (*Leptospermum scoparium*) honey, produced by bees that pollinate the native manuka bush, has high antibacterial activity and is marketed and traded as one of the most medically effective honeys (nutraceutical). The authentication of manuka honey is of great importance; it is a high value honey and it has been reported that much more manuka honey is sold on the retail market than is actually produced, which suggests a high rate of fraud. Kanuka (*Kunzea ericoidis*) honey is one of the major contaminants of manuka honey because the kanuka bush coexists with the manuka bush in New Zealand. The pollen of these two plants is almost identical and indistinguishable by microscopic pollen analysis. Kanuka has different antimicrobial properties to manuka and so it is important to be able to distinguish these two floral sources.

Authentic manuka and kanuka honey samples were collected from hive sites in the North Island (Northland, Wairarapa, Wairoa, Hawkes Bay, East Coast, Taupo and Waikato) of New Zealand and analysed by ultra-performance liquid chromatography – quadrupole time of flight mass spectrometry (UPLC-QToF MS) with multivariate data analysis (MVA).

Using untargeted metabolomics and principal component analysis, reliable discrimination was obtained between manuka and kanuka honeys, as well as between honeys sampled in different regions (Fig. 1). Some of the metabolites that clearly discriminate the sample groups were tentatively identified through database searching using Progenesis MetaScope.

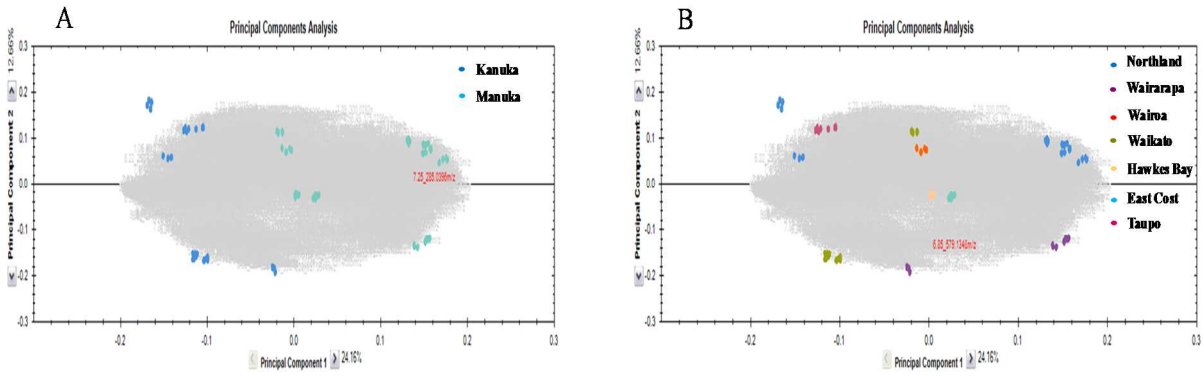

FIG. 1: Principal component analysis performed on kanuka and manuka honeys: (A) floral origin; (B) geographical origin

This is an important development that suggests possible applications of untargeted metabolomics in authentication testing of honeys. The same technique could be applied for other food commodities - we have already investigated this approach for detection of orange juice adulteration with cheaper citrus juices, with the further development of a cheaper and less complex detection method through the identification of selected markers that can be used to differentiate the authentic and adulterated samples using targeted analysis. Both untargeted and targeted metabolomics are included in the suite of methods, with other techniques such as stable isotope analysis, spectroscopic and trace element profiling, that are being developed to support authenticity testing and food traceability systems.

Authentication of Indian citrus fruit/fruit juices by untargeted and targeted metabolomics

Citrus fruits are one of the most important horticultural crops grown in India, and a food commodity that is often targeted for mislabelling worldwide. In the FAO/IAEA Agriculture & Biotechnology Laboratories Activities Report for 2014, we reported on a study to explore the feasibility of using untargeted and targeted analysis to discriminate authentic and adulterated citrus fruits/fruit juices. The results of that study indicated that the targeted analytical method, focusing on the ratios of three of the five identified markers, could be applied for screening for adulteration. However, the method had some shortcomings – for example, using these marker ratios it was not possible to detect addition of Mosambi orange to Jaffa orange juice (although detection of addition of Jaffa to Mosambi was feasible) probably as a result of higher relative concentrations of the markers and the consequent ratios found in Mosambi orange. In 2015, therefore, further research was performed in the FEPL to identify additional biomarkers that can be used to enhance the robustness of the cheaper, more accessible, targeted analytical method.

The study used the same authentic citrus fruit varieties that were used in the previous experiments; Kinnow mandarin (*Citrus nobilis* x *Citrus deliciosa*), Jaffa and Mosambi orange (*Citrus sinensis*), and Redblush grapefruit (*Citrus paradisi*)), obtained from the Soil Microbial Ecology and Environmental Toxicology Laboratory of the University of Delhi, India. These authentic samples were analysed by applying an untargeted method using ultra-performance liquid chromatography – quadrupole time of flight mass spectrometry to identify characteristic markers that could potentially be used to control citrus fruit authenticity. Using this untargeted qualitative approach and applying principal component analysis (PCA) and soft independent modelling by class analogy (SIMCA) it was possible to discriminate between authentic samples and samples adulterated with levels down to 1% of other citrus juices. It was demonstrated that Cooman's plots provide a good means of data visualisation, allowing easy detection of adulterated samples. Fig. 2 displays Cooman's plots of pure and adulterated samples. The plots also show the prediction sets of samples, spiked at various percentages of fruit juices (1, 2, 5 and 10%), applied to the model. The authentic samples lie to the left and to the bottom of the plots, while the adulterated samples lie well beyond the 95% confidence level in the upper right-hand area and do not conform with either of the models.

From the untargeted analysis, nine marker compounds that were influential in the discrimination of the citrus fruits were identified; the five markers from the first study (hesperidin, neohesperidin, naringin, narirutin, and limonin glucoside) and four additional markers (didymin, rhoifolin, isorhoifolin and vicenin-2).

A targeted liquid chromatography-tandem mass spectrometry method was then developed and optimised for the analysis of these markers. The results of the research demonstrated that ratios of: limonin glucoside to hesperidin, narirutin, and didymin; narirutin to hesperidin and vicenin-2; didymin to hesperidin and narirutin; and vicenin-2 to didymin, have the potential to be used to test for authenticity of citrus fruits/fruit juices and to detect adulteration at levels down to 2%. Although less effective than the untargeted analysis, the targeted method is simpler, requires less sophisticated instrumentation and the data is more easily analysed and interpreted making this method potentially more applicable for routine analysis, for example in screening for adulteration.

The results presented in this study showed that untargeted analysis by UPLC-QToF MS with PCA and SIMCA modelling has the potential to be used for the detection of adulteration of citrus juices with similar types of juices at very low levels (down to 1%). The untargeted analysis with multivariate analysis was more effective than targeted analysis of selected markers for this purpose. Cooman's plots provide a good means of data visualisation, allowing easy detection of adulterated samples.

Based on the results from the analysis of the limited number of samples in this study, the use of this methodology could help to improve quality control testing of commercial fruit juices in India and elsewhere, and to increase confidence in the quality of citrus juices on the market, as well as to support exports.

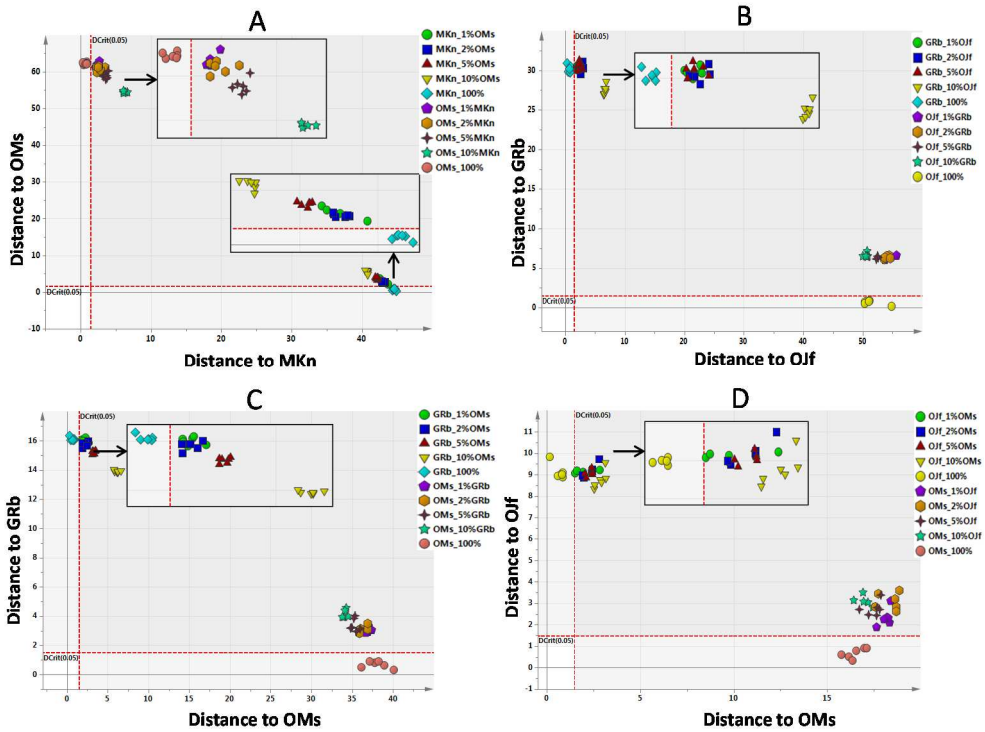

FIG. 2: Cooman's prediction plots for mandarin (cultivar Kinnow, MKn) versus orange (cultivar Mosambi, OMs) (A), grapefruit (cultivar Redblush, GRb) versus orange (cultivar Jaffa, OJf) (B) and Mosambi, OMs) (C), and orange Jaffa versus orange Mosambi (D). The authentic samples lie to the left or below the dotted lines, which represent the 95% confidence levels, and the adulterated samples (1, 2, 5 and 10%) lie in the upper right quadrant in each plot.

Hydrogen Isotope Ratio Analysis in Tea

One approach to increase the accessibility and cost-effectiveness of methods for food authenticity or the verification of food origin is to reduce the reliance on data from authentic samples by making use of our understanding of how environmental factors influence the stable isotope ratios of the food product. There are many 'drivers' of isotopic composition, the best understood being the ratio of hydrogen isotopes in rainfall. The IAEA, through its Global Network of Isotopes in Precipitation (GNIP), has collected a very large database of rainfall data that reveal systematic patterns in global distribution of isotope ratios of hydrogen (and oxygen). It has been found that these patterns are transferred into the food, and therefore measurements of hydrogen and oxygen isotope ratios provide a link from the food product to its geographical origin (Fig. 3).

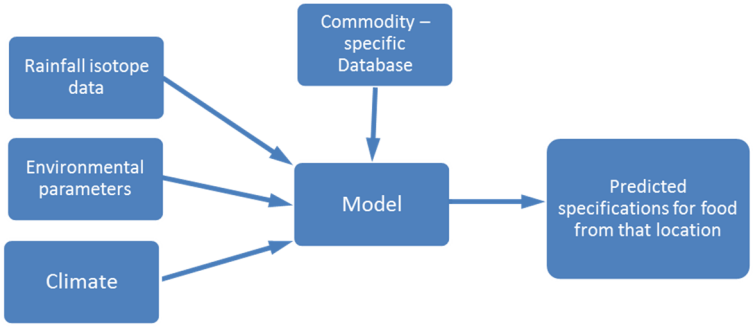

FIG. 3: Schematic of environmental factors used to develop a predictive model for geographical origin of food.

A complication that arises is that not all the hydrogen in food behaves in a similar way. Hydrogen that is bound to carbon atoms is strongly held and incorporates the isotope signal from its origin. However, hydrogen bound to O or N atoms, e.g. in carboxylic or amino acids, is weakly bound and can exchange with ambient water vapour over short timescales. Therefore at least some of the hydrogen isotope signal measured will come from the water vapour encountered

post-harvest, including in the laboratory. Equilibration methods have been developed to account for this exchangeable portion but they are time-consuming and require considerable skill and specialised equipment to implement. The FEPL has adapted a method that targets only non-exchangeable hydrogen by producing methyl iodide (CH_3I) from a nucleophilic substitution reaction with the methoxyl groups on pectin extracted from the food. This is demonstrated in the schematic below (Fig. 4). Pectin is extracted from tea (Fig. 4A) and dried overnight. An aliquot is reacted with hydro-iodic acid (HI) for 30 min at 110°C to produce CH_3I (Fig. 4B). The CH_3I gas is injected into the carrier stream of an elemental analyser where it is pyrolysed to produce H_2 gas for isotopic analysis (Fig. 4C).

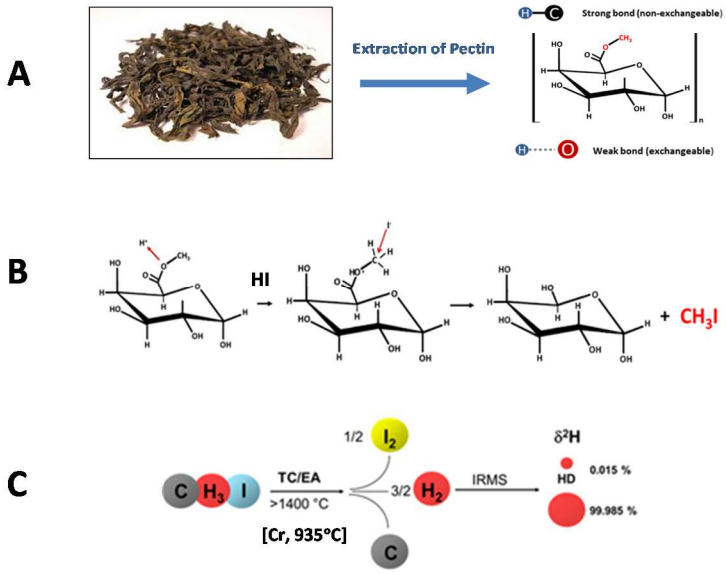

FIG. 4: Extraction of non-exchangeable hydrogen for isotopic analysis

Initially the pyrolysis was conducted using a high-temperature system (TC/EA at 1400°C) with glassy carbon as the reductant. It was found that 5 g of tea provided 150 mg of pectin; enough for triplicate preparations of CH_3I. The precision of δ^2H measurements was comparable to conventional techniques - 2‰ achieved from repeat injections from the same vial. Repeatability between preparations of the same pectin was 3‰. The time taken for analysis was 2 hours for extraction, overnight drying in a freeze-dryer, 45 minutes for preparation of CH_3I and 10 minutes per sample for mass spectrometry. This compares well with the room temperature equilibration (10 days plus analysis time) and is similar to the steam equilibration process.

While these results are very encouraging, the method still relies on a specialised high-temperature conversion system. Further experiments were conducted where we replaced the glassy carbon reactor with a quartz reactor filled with chromium powder. The use of Cr has several advantages; the cost per reactor is <10% of a glassy carbon reactor, the reduction to H_2 occurs at 935°C, so is amenable to a standard elemental analyser, and the I_2 by-product is trapped on the Cr so there is no need for further traps to purify the gas stream prior to admission to the mass spectrometer. The testing of the Cr system is still underway but initial results indicate better reproducibility than was achieved with the glassy carbon.

EU 7th Framework project 'FoodIntegrity'

The FEPL is a research partner in the multi-national Integrated Project, 'FoodIntegrity', funded under the EU 7th Framework mechanism, which commenced in 2014.

Providing assurance to consumers and other stakeholders about the safety, authenticity and quality of European food (integrity) is of prime importance in adding value to the European agri-food economy. The integrity of European foods is under constant threat from fraudulently labelled imitations that try to exploit that added value. The FoodIntegrity project will directly address this issue and will be an international focal point for harmonisation and exploitation of research and technology for insuring the integrity of European food. Comprising an inner core of project participants from industry, academia, research institutes, technology providers and a global network of stakeholders, FoodIntegrity will rationalise and harmonise capability to provide a coherent structure and process for assuring the food supply.

The 2nd annual meeting and conference of the project was hosted by AZTI Tecnalia in Bilbao, Spain, from 24-27 March 2015. The FEPL produced outputs under three work packages (WP) within the project: WP1, Food Integrity network; WP2, Knowledge Base; and WP 10, Industrial Integration. The FoodIntegrity conference had 150 registered participants, who engaged in lively discussion on the conference topics. Two sessions were chaired by the FEPL Head, who also gave a presentation on information and technology transfer in the satellite workshop, 'Formation of a Network of Excellence for Food Authenticity Analysis - Key Challenges'.

Participation in the FoodIntegrity project will lead to outcomes that are of direct benefit to IAEA Member States and in line with the Food and Environmental Protection sub-programme's objectives. Participation in the work package meetings, the conference and the workshops was extremely beneficial in consolidating and expanding collaboration and cooperation with other institutes, and the information and knowledge exchange was at the very apex of the science in this field of work.

Coordinated research

The FEPL coordinates and provides technical input to two coordinated research projects (CRPs) in the fields of food authenticity and traceability. The project 'Implementation of Nuclear Techniques to Improve Food Traceability' commenced in 2011 and is due to end in 2016. The project has 16 participating laboratories in 15 countries. The third research coordination meeting for this project was held in Kampala, Uganda from 26-30 October 2015. Progress by most partners was as expected according to the work plans.

This CRP has made excellent progress in the sampling and analysis aspects of functional methodology to underpin traceability systems. This has involved achieving good understanding of commodity-specific production systems. The protocols developed have proven to be robust and most of the QC issues have been addressed. The analytical procedures developed through the CRP are ready for wider dissemination among Member States. The datasets generated need to be housed in a publically-accessible database that also includes (or is linked to) the vast amount of metadata that is available (meteorological data, geological and land usage maps, etc.).

The overall aim of the CRP is to develop systems based on nuclear technologies that contribute to food safety and traceability by independently verifying the authenticity of food and natural commodities. The project has achieved the first stage of this by demonstrating the applicability of the techniques to a wide range of foodstuffs. The protocols and databases developed are foundational to the future development of food control systems. Each of the contract holders has demonstrated the requisite competence in nuclear and related techniques and so capacity for this technology has been built in participating Member States.

Awareness of the contribution that nuclear techniques can make to food safety and trade has been raised within the Member States. One of the main barriers to entry for this technology is simply that stakeholders are unaware of its potential and capabilities for food traceability and authentication. Publicity around the CRP as well as individual presentations using resources developed in the CRP have raised awareness and interest among food producers, regulators and government officials.

Key outputs and impacts include:

- Extensive collections of authentic food samples from Member States gathered by project partners or trusted national representatives.
- Corresponding databases of isotopic, elemental, spectroscopic, genetic and chemical measurements. Preliminary GIS maps containing the spatial variability of the isotope signature of food commodities.
- Standard Operating Procedures (including QA and QC) and harmonized protocols for the determination of the provenance of food, using nuclear and other complementary techniques.
- Ten publications in peer reviewed scientific food and agriculture journals.

- Fifteen oral and poster communications in national and international food science and related symposia.
- Collaboration with and methods submitted to Member State National Food Control authorities for consideration.
- Collaboration with both national food industry and food association partners and international academic and public sector research partners.
- Seven associated Master and five associated PhD studentships that have contributed to, and benefited from association with, the CRP.

The second project in this field of work, 'Accessible Technologies for the Verification of Origin of Dairy Products as an Example Control System to Enhance Global Trade and Food Safety' has 15 participating laboratories in 15 countries. Research is proceeding as planned in this project. Unfortunately, the research coordination planned for 2015 had to be postponed until 2016 to allow replacement of the project officer.

Control of Residues and Contaminants in Food

The control of unwanted chemicals in food, such as residues of veterinary drugs or pesticides used in food production, or natural contaminants such as mycotoxins, remains an area of high importance to Member States, as demonstrated by the high number of requests for assistance through the IAEA technical cooperation programme, through FAO, and requests directly from Member States to the FEPL. Activities performed in FEPL to underpin capacity building in this area include applied research on analytical methodology to enable Member States to perform targeted risk assessment, and the development or adaptation and validation of analytical methods for the detection, quantification and control of residues and contaminants.

Method validation for selected pesticides in potato by gas chromatography coupled to tandem mass spectrometry

Potatoes are an important staple food all over the world. To protect the crop from various diseases farmers apply a range of regulated pesticide formulations, which can sometimes leave residues in the crop. To help ensure safe food for consumers it is important to apply end control testing to agricultural products. As part of an initiative under the "Red Analitica de Latinoamérica y el Caribe" (RALACA) network, the FAO/IAEA's FEPL contributed to the validation of a multi-residue method for 85 pesticides in potato. The method includes the pesticides that are most frequently used in potato production. The aim of the study was to validate the method according to the Codex Alimentarius Guidelines on Good Laboratory Practice in Pesticide Residue Analysis (CAC/GL 40-1993) using gas chromatography coupled to tandem mass spectrometry (GC-MSMS). Within-laboratory method validation experiments were conducted to provide evidence that the method is fit-for-purpose. The method was validated using two different sample preparation options. One was the original QuEChERS method using acetonitrile as the extraction solvent and the salts from an Agilent QuEChERS kit. The second was an IAEA modified QuEChERS method using ethyl acetate as the extraction solvent and clean up using QuEChERS salts. The sample preparation steps are outlined in Fig. 5. Detection was carried out using GC-MSMS.

The instrument acquisition method for the GC-MSMS was based on Agilent's Pesticide Analyser configuration for a 7000C Triple Quadrupole, and was optimised using the Agilent RTL MRM data base, resulting in a 22-minute run time.

The method performance was characterized in terms of its scope, specificity, accuracy, sensitivity, repeatability, within-laboratory reproducibility and robustness. The experimental design for validation involved the analysis of 85 pesticides, belonging to several different chemical classes, at three spiking levels: 10 µg kg^{-1} (the reporting limit), 20 µg kg^{-1} and 40 µg kg^{-1}. Six replicate samples were spiked at each level and the study was repeated three times with both methods. Matrix

matched calibration was used to compensate for matrix effects, with the addition of an internal standard to correct for possible chromatographic effects.

The calibration was linear in the range of 5 – 100 µg kg^{-1}. Ion ratios for compound confirmation were within 30% of the average ion ratio values derived from the calibration curve, complying with the quality criteria according to analytical guidelines (SANCO/12571/2013). Similar recoveries were obtained with both extraction procedures, with a few exceptions. Most of the pesticides had recoveries and relative standard deviations within the CODEX acceptable range of 60-120% and 20%, respectively.

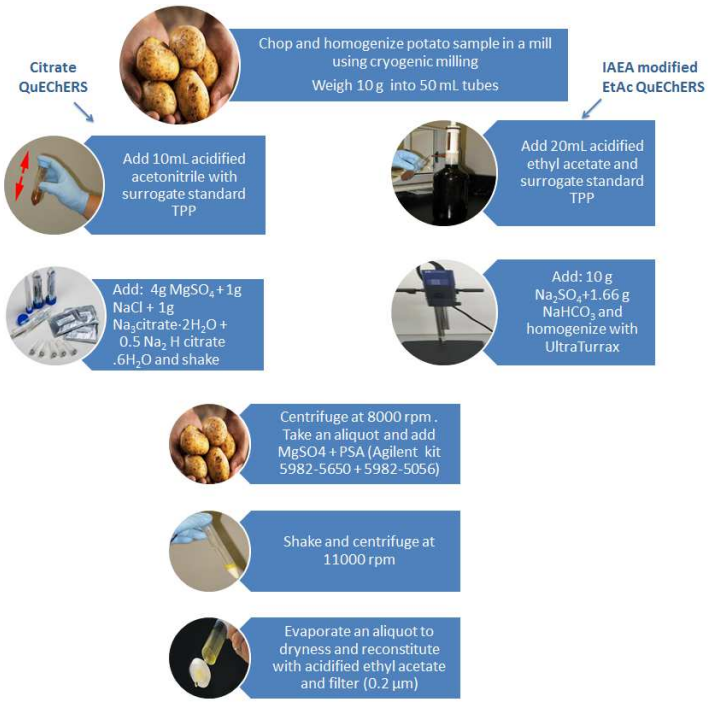

FIG. 5: General description of the method

The sample extracts analysed by GC-MSMS were also analysed using a single quadrupole mass selective detector (GC-MSD). Figure 6 shows a comparison between the recoveries and relative standard deviations of 34 pesticides at 10 µg kg^{-1} detected using the two different detection systems. Both instruments had good sensitivity for a number of compounds; however, the GC-MSMS method was more robust due to the increased confirmatory power and lower variability of results.

The method is quick, robust and relatively cheap. The IAEA modified QuEChERS method using ethyl acetate as extraction solvent provides a cheaper alternative in routine analysis using gas chromatographic detection. Future work will include the extension of the method to additional pesticides more amenable to liquid chromatography-tandem mass spectrometry, giving the possibility of using a single extraction method and two detection methods to cover a broad range of pesticides.

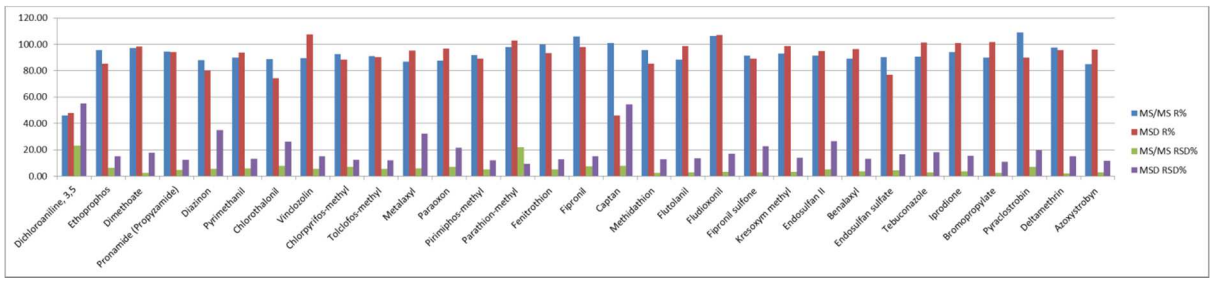

FIG. 6: Recoveries and relative standard deviations of 34 pesticides at 10 µg kg^{-1} level using single quadrupole (MSD) and triple quadrupole (MSMS) mass spectrometric systems

Collaboration with Uruguay – visiting scientists in FEPL

The FEPL started a technical collaboration in 2015 with the University of the Republic (UdelaR), Montevideo, Uruguay, to collaboratively study the validation of methods of analysis for pesticide residues in difficult matrices such as medicinal plants. The objective of this work is to enable Member States to monitor contamination in consumer products and to protect national and regional

value markets. Ms Natalia Besil and Prof. Veronica Cesio visited the FEPL to work on the validation of a method for the detection of 41 pesticides in *Peumus boldus* (boldo) using GC-MSMS. Boldo is a medicinal plant common in Chile that is included in the most recent Edition of the European Pharmacopoeia and commonly used as a herbal tea to aid digestion and prevent stomach ailments. It is a natural forest plant and is also cultivated to meet the high demand from all over the world. Boldo is therefore of great economic interest as an agricultural product that constitutes a valuable market opportunity.

The boldo matrix is very complex because of the high content of secondary metabolites with similar physicochemical properties to the pesticides under study, interfering with their extraction and detection. Previous studies in Uruguay identified the optimum sample preparation method as a modified, citrate-buffered QuEChERS method (Fig. 7).

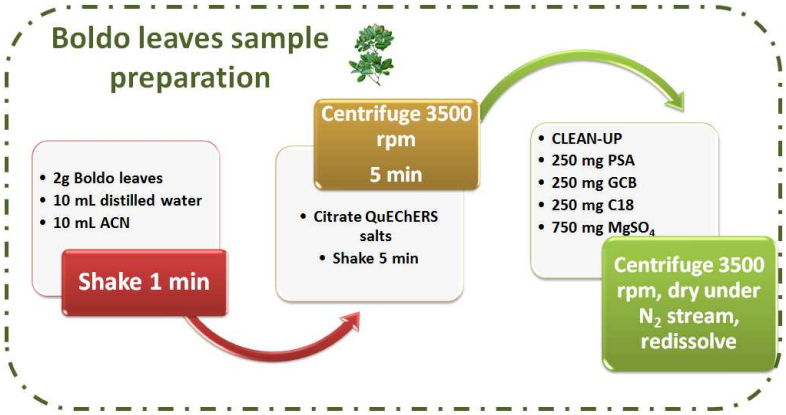

FIG. 7: Sample preparation for boldo leaves

A specific method was also developed for the analysis of pesticide residues in the boldo infusion. Homogenized boldo leaves were infused for 10 minutes with warm water, following the same procedure adopted for home tea preparation, and filtered. A portion of the infusion was extracted with ethyl acetate and prepared according to the procedure in Fig. 8.

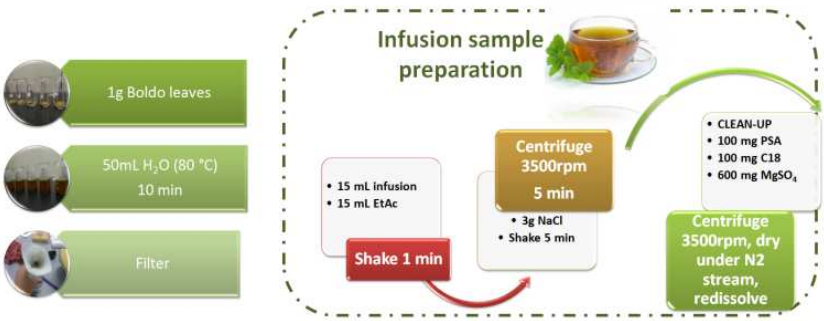

FIG. 8: Sample preparation for boldo infusion

Based on this sample preparation strategy, the method was tested and validated using GC-MSMS. The instrument detection method was modified from Agilent's Analyser configuration.

Significant matrix effects were observed at the retention times where the pesticides under study eluted. It is hypothesised that the matrix effects in boldo are due to components such as ascaridol and boldine that have high reactivity in the GC injection port. Further studies using high resolution mass spectrometry are needed to understand the behaviour of boldo extracts in GC analysis.

The method performance was acceptable for all pesticides tested at 50 µg kg^{-1}. At 10 µg kg^{-1} about one third of the pesticides under study gave poor recovery for the boldo infusion, indicating that further work is required to fully develop this method.

The methods were applied to study the transfer of pesticide residues from the leaves to the infusion. Most of the pesticides under study showed less than 40% transfer (Fig. 9). However, some compounds, including carbofuran, dimethoate, metalaxyl and oxadixyl showed a higher percentage transfer to the infusion. Depending on the initial concentration, adverse health effects could possibly

occur due to these pesticide residues. Therefore, the monitoring of the contamination levels in herbal medicinal plants is important to be able to protect consumers.

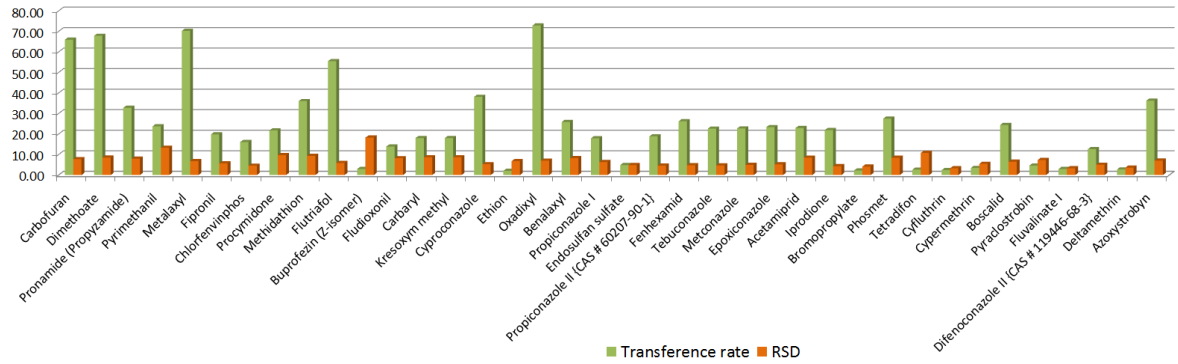

FIG. 9: Percentage transfer of pesticides into the infusion

Coordinated research

The FEPL participates in the CRP 'Development and Strengthening of Radio-Analytical and Complementary Techniques to Control Residues of Veterinary Drugs and Related Chemicals in Aquaculture Products', which commenced in 2015. This project includes 15 laboratories in 14 countries, plus the FEPL.

Aquaculture is becoming more widespread for the inexpensive and intensive production of protein rich foods. In the period 2000–2012, intensive aquaculture production increased at an average annual rate of 6.2% from 32.4 million to 66.6 million tons globally. Agrochemical inputs such as veterinary pharmaceuticals and related substances are required to control aquaculture-related diseases and improve yields. Residues of such inputs, plus unintended natural toxins in aquaculture products and feeds, as well as contaminants at production sites, pose public and environmental health risks and must be controlled. This calls for robust national regulatory frameworks, underpinned by competent laboratories, to safeguard consumers, optimise aquaculture production and enhance international trade in aquaculture products. Nuclear and isotopic techniques can play an important role. Research is needed on analytical methods that will strengthen laboratory performance, and to better understand the contamination of aquaculture production sites, which has potential public and environmental health implications.

Through coordinated research, this project aims at strengthening Member State analytical laboratories and national chemical residue monitoring programmes, thus contributing to the improvement of food safety, better aquaculture production and management practices as well as enhancement of trade in aquaculture products. New analytical methods will be developed, including improved environmentally friendly sample preparation techniques, validated and transferred amongst Member States laboratories. The FEPL is involved in developing methods and validation protocols for transfer and in-house validation in partner laboratories, and the provision of advice and guidance on method development and validation.

Dissemination of Research Results

The methods developed or adapted and validated in the FEPL are made available to Member States through various mechanisms, including training workshops, publications in the scientific literature and via the internet. The 'Food Contaminant and Residue Information System' (FCRIS, http://nucleus.iaea.org/fcris/) provides a wealth of useful data on food contaminants and residues and includes analytical method databases, which are continually updated with methods developed in the FEPL as well as others submitted by laboratories in Member States. The methods databases for veterinary drug residues and for pesticide residues were developed in response to requests from the Codex Committees on Residues of Veterinary Drugs in Food and on Pesticide Residues.

Conferences

- 7th International Symposium on Recent Advances in Food Analysis (RAFA), Prague, Czech Republic, 3-6 November 2015. This symposium is a biennial event. The 7th symposium covered a wide range of topics, including recent advances in analytical and bioanalytical technologies, allergens, novel foods and supplements, emerging food-related issues, risk assessment, food authenticity and fraud, and the analysis of nanoparticles, residues and natural contaminants. The symposium had approximately 800 registered participants from more than 65 countries. Ms Zora Jandrić (FEPL) presented a poster entitled 'Differentiation of manuka and kanuka honeys by mass spectrometry and chemometrics'. This research, performed in the FEPL under the project 'Traceability to improve food safety and quality and enhance international trade', represented an important step forward in the development of methodology to protect product authenticity and combat fraud, which is a very costly problem in many countries. Two posters on collaborative work with FEPL were presented by project counterparts from the Laboratorio de Bromatología in Montevideo, Uruguay.
- 'FoodIntegrity' Open Days, Prague, Czech Republic, 4-5 November 2015. The RAFA symposium programme also included two open-day sessions of the EU 7th Framework project 'FoodIntegrity, in which FEPL is involved in the work packages; 'Food Integrity Network', 'Knowledge Base' and 'Industrial Integration'. Mr Cannavan represented FEPL in both open-day sessions.
- EXPO 2015 Conference, 'Fighting Food Crime - Enforcing Food Safety', Milan, Italy, 9-10 July 2015. The two-day conference was held by the Italian national competent authorities for food safety. The programme included a plenary session comprising a number of presentations by the Italian competent authorities; representatives of countries from Europe (session 1), the Americas (session 2), Asia and the Pacific (session 3); and international cooperation to combat food fraud (session 4). Mr. Cannavan was invited to give a presentation in session 4 of the plenary session, representing the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. Mr. Cannavan presented the work done at the FEPL in developing, validating and transferring isotopic and complementary methods for food authentication and tracing of origin, in the context of holistic food control systems.
- 5th Latin American Pesticide Residue Workshop (LAPRW), Santiago, Chile, 10-13 May 2015. The fifth workshop in the series of biennial LAPRWs had approximately 350 participants from more than 30 countries. The workshop covered a wide range of topics including methods of analysis for pesticides, sample preparation and clean up procedures, guidelines on analytical quality control and validation, pesticide regulations, environmental risk assessment and monitoring programmes for pesticide residues. The first RALACA general meeting and several vendor workshops were also included in the agenda. Ms Britt Maestroni (FEPL), gave an oral presentation entitled 'Method validation for pesticide residue testing: are there still issues and challenges to tackle?'. Several posters were presented by project counterparts and former trainees at Seibersdorf, including representatives from Argentina, Chile and Peru.
- UK Food and Environment Research Agency (FERA) Science Conference, Sand Hutton, UK, 27 January 2015. The Head of the FEPL was invited to give a plenary talk at the annual FERA Science Conference. FERA is an applied research agency with the vision to be the leading provider of science-based solutions, evidence and advice across the agri-food supply chain. The institute is a very important collaborator with IAEA, providing expertise in both research and capacity building for IAEA projects, and serving as coordinator for several extrabudgetary projects through which the FEPL receives funding. The aim of this 1-day event was to promote cross-disciplinary interaction by bringing together colleagues to share knowledge and discuss the latest scientific trends. Mr Cannavan gave a presentation on 'The Application of Chemical Measurement Techniques to Support Food Traceability and Authenticity'.

- American Society for Mass Spectrometry (ASMS) Sanibel Conference, 22-25 January 2015, Florida, USA. Mr Russell Frew (FEPL) gave an invited oral presentation at the 2015 ASMS conference on the role of nuclear techniques in food authentication and traceability, and on the activities of FEPL in particular. Topics included the development of analytical and sampling strategies for implementation under the CRP 'Accessible Technologies for the Verification of Origin of Dairy Products as an Example Control System to Enhance Global Trade and Food Safety' and the development of geospatial modelling approaches to the datasets being generated.
- The FEPL was represented by the Laboratory Head on the Scientific and Publication committees for the Saskatoon International Workshop of Validation and Regulatory Analysis, which was held in Calgary, Canada, 16-19 June 2015. The FEPL Head is also Chair of the Scientific Committee for the EuroResidue VIII Conference on Residues of Veterinary Drugs in Food, to be held in Egmond aan Zee, The Netherlands, 23-25 May 2016, preparatory work for which was ongoing in 2015.

CAPACITY BUILDING

The FEPL provided technical management for twenty national and five regional TCPs in 2015. Analytical methods and technology packages were transferred and applied through the TCPs and through training workshops held in Member States using extra-budgetary funding. More than 160 scientists, analytical chemists, laboratory personnel and food inspectors from more than 50 countries were trained through these workshops. Each workshop was designed with an individual focus, but all were within the framework of food safety and quality and included the protection of the integrity of the food supply chain as a holistic process, involving multiple stakeholders and requiring the application and integration of different analytical methods and technologies. The workshops provided a forum for interdisciplinary networking between stakeholders in the "farm-to-fork" food chain and fostered the formation of a global network.

FAO/IAEA Regional workshop on 'Method validation for pesticide residue testing'

The FEPL, in collaboration with the Regional Office of the Food and Agriculture Organization of the United Nations (FAO) in Santiago, Chile, organized a training workshop on 'Method validation for pesticide residue testing' from 6-8 May in Santiago, Chile. The training workshop was attended by 41 analytical chemists from Argentina, Brazil, Chile, Colombia, Costa Rica, Guatemala, Panama, Paraguay, Peru and Uruguay. The training covered theoretical aspects related to the validation of analytical methods for pesticide residue testing including in house and collaborative trial approaches, statistics, quality assurance and control measures, uncertainty estimation, robustness testing, analytical methods, planning of experiments and data analysis.

Participants in the workshop 'Method validation for pesticide residue testing', Santiago, Chile

The participants were satisfied with the training: 70% of the participants indicated that the scientific content of the workshop was excellent and 26% that it was good, and had met their objectives. The workshop provided an excellent opportunity to interchange experiences,

methodologies and practical applications for method validation in pesticide residue testing. The group discussions helped to identify current important issues and challenges and to improve the technical competence of analytical laboratories.

FAO-IAEA/GFSP/UNIDO Training Workshop on 'Food Safety, Quality and Traceability'

The FAO and the IAEA, through the FEPL, in collaboration with the Global Food Safety Partnership (GFSP) and the United Nations Industrial Development Organization (UNIDO), organized a two-part train-the-trainers workshop on 'Food Safety, Quality and Traceability' in Viet Nam, in Ho Chi Minh City from 9-13 November and in Hanoi from 16-20 November, 2015. The workshop was attended by 49 participants representing 11 countries in the Asia Pacific and Middle East region. The workshop was one in a series under the Peaceful Uses Initiative (PUI) Project on 'Sustainability of capacity building activities to improve food safety and quality through nuclear technology and networking', which is funded by the USA. A key objective was to raise awareness of the requirements for effective food control systems to protect the integrity of the food supply chain, with a focus on the role of the analytical laboratory.

Participants in the training workshop in Viet Nam

The first week of the workshop was hosted by the Quality Assurance and Testing Center 3 (Quatest 3), which is a science-technological organization under the Directorate for Standards, Metrology and Quality of the Ministry of Science and Technology of Viet Nam. The programme included lectures on food control systems and the role of the different stakeholders in the farm-to-fork food chain, international guidelines and regulations for consumer protection and international trade, an overview of isotopic and other complementary analytical technologies for food authentication and traceability, food contaminant analysis and sample preparation options, method validation, analytical requirements, and an overview of advanced analytical instrumentation. The second part of the workshop was laboratory-based and was hosted by the National Institute for Food Control (NIFC) in Hanoi. The laboratory training focused on various aspects of mycotoxin analysis. Extensive practical laboratory training on methods to detect mycotoxins in food and feed took place at the NIFC laboratory covering topics such as lateral flow tests for aflatoxins, enzyme linked immunosorbent assay (ELISA), immunoaffinity column clean-up, standard preparation, and liquid chromatography coupled with fluorescence detection and mass spectrometric detection. The importance of establishing quality systems and applying quality control and quality assurance measures in the laboratories was stressed. The participants of the second week of the workshop received a laboratory manual on methods for the determination of mycotoxins in food, prepared by the International Food Safety Training Laboratory of the University of Maryland, JIFSAN, in collaboration with US FDA and Waters Corporation.

Participants in the training workshop in Viet Nam

The training workshop was well received by the participants. A final evaluation questionnaire indicated that all participants were happy with the workshop and that 82% judged they will be able to apply the knowledge acquired once they are back in their laboratory. One important result of the workshop was the formation of an informal regional network of scientists from the Asia Pacific Region and the Middle East. Recommendations to the IAEA from the workshop participants included the establishment of a follow-up programme and an impact assessment to further consolidate the interdisciplinary networking.

FAO/IAEA Workshop on Food Safety - Challenges for Developing Countries

Effective food control systems are needed to ensure a safe and wholesome food supply on a global basis. One key element of such a system is the provision of feedback on the effectiveness of agricultural practices in producing food that is safe and meets requirements for international trade. In this regard analytical laboratories, to underpin monitoring and surveillance schemes, are essential. Testing of agricultural products is important to maintain consumer confidence, ensure food safety, and facilitate international trade and the provision of safe food supplies.

Implementing these testing schemes is challenging, especially for developing countries, and requires strong networking, human resource development, research and capacity building in the field of food control systems. To help Member States in this regard the Joint FAO/IAEA Division held a workshop on 'Food Safety - Challenges for Developing Countries' in conjunction with the 7th International Symposium on Recent Advances in Food Analysis (RAFA), in Prague, Czech Republic, 3-6 November 2015. Workshop participants also participated in the full RAFA symposium.

The purpose of the workshop was to identify problems and issues of high importance and to provide information and guidance on research and capacity building in the field of food control systems. The workshop had more than 70 participants from more than 30 countries.

Mr Cannavan moderates the panel discussion session

Mr Andrew Cannavan, head of the FEPL, chaired and opened the meeting. Presentations were given by Mr Ihsan Ihsanullah (Nuclear Institute for Food & Agriculture, Pakistan), Mr Alphonse Yakoro (National Public Health Laboratory, Burkina Faso), Ms Veronica Cesio (UdelaR, Uruguay), Mr Bruno Le Bizec (ONIRIS-LABERCA, France) and Ms Janie Dubois (International Food Safety Training Laboratory, JIFSAN, USA).

Following the presentations, the lecturers formed an expert panel for an interactive discussion session with the workshop participants, which was moderated by Mr Cannavan, assisted by Ms Zora Jandrić.

The workshop provided an excellent opportunity for developing country scientists to network and develop working collaborations with participants in RAFA. This networking will enhance the sustainability of the control systems in Member States and will help to harmonise the approach to food safety control internationally.

Contributions to External Training

FEPL contributed to two training courses run by external organisations in 2015.

Mr Russell Frew was invited to lecture in a training course on 'Innovative technologies to enhance the traceability of the food chain', which was organised by the Centre International de Hautes Etudes Agronomiques Mediterraneennes (CIHEAM) of the Mediterranean Agronomic Institute of Zaragoza, in Zaragoza, Spain, from 25-27 March 2015. The course had 34 delegates from 12 Member States (Albania, Algeria, Brazil, Egypt, Italy, Lebanon, Morocco, Portugal, Senegal, Spain, Tunisia and Turkey).

Mr Frew provided lectures on geochemical and complementary technologies, including the application of nuclear techniques for verifying the authenticity and origin of natural products including food, other geochemical techniques to verify the origin of food, and the role of complimentary techniques including metabolomics and spectroscopy in food traceability and authenticity systems.

Ms Britt Maestroni was invited to present an introductory lecture at a Summer School on 'Food Safety and Food Security: a Multilevel Educational Perspective', organized by the University of Brescia, Italy, under the patronage of EXPO 2015. The goal was to provide students with a multidisciplinary perspective on the societal and scientific challenges of food security and food safety. Ms Maestroni's lecture highlighted the central role played by the analytical laboratory in providing end product testing and advice in the context of food control systems. The lecture was attended by twenty university students from Italy.

The RALACA Laboratory Network

The 'Red Analitica de Latinoamérica y el Caribe' (RALACA) is a non-profit network of laboratories and associated institutions in Latin America and Caribbean countries that aims to enhance regional capabilities for food safety and environmental sustainability (http://red-ralaca.net). The network was initiated and established with FEPL assistance in March 2012, with nine laboratories initially involved. Today RALACA encompasses more than 50 laboratories and institutions in 21 countries, with new members from Brazil, Ecuador, El Salvador and Guatemala since May 2015. The network held its first general meeting on the 11th of May 2015 in Santiago, Chile, on the occasion of the 5th Latin American Pesticide Residue Workshop. The meeting was attended by 80 participants. RALACA operates through a governing board and a number of committees. The FEPL maintains input and assists with coordination of the network through membership in the board. In 2015 RALACA organized a number of training courses and meetings, sponsored by the IAEA and coordinated by FEPL under the regional project RLA7019. Three training courses were held, one on bio-indication of pesticide contamination in aquatic and terrestrial environments using radiometric techniques from 20-31 July in Brazil, one on statistics and modelling from 11-22 May in Chile and one on scientific communication from 11-15 May in Argentina. RALACA members also attended a meeting on validation of the biological monitoring working party (BMWP) index for neo-tropical streams from 8-11 September in Costa Rica, and on Soil & Water Assessment Tool (SWAT) modelling from 14-25 September in Costa Rica.

The Global Food Safety Partnership

The Global Food Safety Partnership (GFSP) is a public-private partnership dedicated to food safety capacity building. The main GFSP objective is to support improved food safety systems as demonstrated by enhanced agri-food value chains for economic growth and improved public health outcomes in developing and middle income countries. The GFSP approach is intended to fill a gap whereby food safety initiatives would be better coordinated and accessible to improve impact.

The FEPL interacted with the Laboratory Capacity Working Group of the GFSP in 2015 in the organisation and implementation of two workshops; the workshop on 'Food Safety, Quality and Traceability' in Viet Nam, in Ho Chi Minh City from 9-13 November and in Hanoi from 16-20 November, 2015 (which also had input from UNIDO), and the FAO/IAEA Workshop on Food Safety

- Challenges for Developing Countries, held in conjunction with the RAFA Symposium in Prague, Czech Republic on 3 November 2015, at which Ms Janie Dubois, Chair of the Laboratory Capacity Working Group gave a presentation and participated in panel discussions.

Fellowships, Scientific Visitors and Interns

The FEPL had a total of six interns during 2015.

Ms Victoria Ochoa's internship ended on March 31, 2015 after one year at FEPL under the supervision of Ms Britt Maestroni and Mr Andrew Cannavan. Her work contributed to the development, adaptation and validation of nuclear and related techniques to improve food safety and environmental sustainability. Her responsibilities included performing laboratory tasks such as sample preparation and extraction for the analysis of pesticides in food and soil by gas chromatography coupled to mass spectrometry, the use of radiotracers to estimate soil sorption parameters, analysis and interpretation of laboratory data and assisting in the preparation of scientific publications. She was involved in the preparation of training materials and laboratory practical sessions for a workshop on quality assurance and quality control procedures to ensure food quality and safety.

Ms Yasmin Leithner's internship in FEPL also ended in March 2015. Yasmin joined the laboratory in July 2014. Her work included support in the development and application of analytical methods for food authenticity and traceability by stable isotope analysis, covering a range of food types. She contributed to the development of a number of methods, including methods for carbon and hydrogen isotopic analysis for the authenticity testing of food samples in connection with research partners in Sri Lanka and Pakistan, facilitating parallel research in those countries. She gained experience in various bench and instrumental techniques, including mass spectrometric techniques for stable isotope measurements and for food contaminant detection.

Ms Agneta Krukle was an intern in the FEPL from April until October 2015, following a short internship in the Soil and Water Management and Crop Nutrition Laboratory. During her internship, Agneta worked mainly on the development of a method for the analysis of pesticide residues in potato.

Ms Hanna Zakala joined FEPL from the National University of Food Technologies, Ukraine, in July 2015. Hanna will work on a variety of projects during her internship, and is currently involved in metabolomics analysis of food products for food authenticity studies, under the supervision of Zora Jandrić.

Ms Helene Muehlehner joined FEPL for a two-month internship in August, from the University of Natural Resources and Life Sciences, Vienna, Austria, where she was studying environmental and bio-resources management. She worked mainly on stable isotope measurements and related analytical techniques for food traceability and authenticity.

Ms Valeria Avossa commenced her internship in December 2015. Valeria studied in Italy at the Università di Napoli Federico II, Napoli, and the Università di Bologna. She will also gain experience in a variety of projects, mainly working on pesticide residue analysis under the supervision of Britt Maestroni.

The FEPL also hosted three visiting scientists in 2015. Prof. Veronica Cesio and Ms Natalia Besil, from the University of the Republic, Montevideo, Uruguay, (UdelaR), worked with FEPL staff on the validation of methods of analysis for pesticide residues in difficult matrices such as medicinal plants. Dr Celine Lesueur, Head of R&D at LVA GmbH (Lebensmittelversuchsanstalt – Food Research Institute), Austria, worked on various occasions with FEPL staff on the development and optimisation of methods for pesticide residues analysis.

PUBLICATIONS

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JANDRIĆ, Z., FREW, R.D., FERNANDEZ-CEDI, L.N., CANNAVAN, A. (2105). An investigative study on discrimination of honey of various floral and geographical origins using UPLC-QToF MS and multivariate data analysis. Food Control, in press, published online: doi:10.1016/j.foodcont.2015.10.010.

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HOLDER, P.W., FREW, R., VAN HALE, R. (2015). The geographic origin of an intercepted biosecurity pest beetle assigned using hydrogen stable isotopes. Journal of Economic Entomology (doi: 10.1093/jee/tou097).

CANNAVAN, A., FREW, R., JANDRIĆ, Z. (2015). Chemical measurement techniques to support food traceability and authenticity. Book of abstracts of the FERA Science Conference, Sand Hutton, UK, 27 January 2015, 2-3.

EXTERNAL COLLABORATIONS AND PARTNERSHIPS

Institution	Topic
The Food and Environment Research Agency (fera), UK	Research on food authenticity and traceability EU project 'FoodIntegrity' EU project 'Authent-NET'
Centro de Contaminacion Ambiental (CICA), University of Costa Rica (UCR), Costa Rica	IAEA Collaborating Centre for eLearning and Accelerated Capacity Building for Food and Environmental Protection (EACB)
Austrian Agency for Health and Food Safety (AGES), Austria	Collaboration on accelerated capacity building for risk analysis and contaminants in food
Austrian Institute of Technology, Austria	Collaboration on nuclear techniques for research into interactions between environmental/food contamination Collaboration on the use of stable isotope measurements for traceability of foods and animals
Global Food Security Institute, Queen's University Belfast, UK	Research and method development activities for food contaminants and food traceability
ASSET Centre, Queen's University Belfast, UK	Research activities in isotope-ratio methods for food traceability
Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA)	Training for Member State scientists and regulators on food safety and quality
Agilent Technologies, PA, USA	
Thermo Scientific, MA, USA	
Waters Corp., MA, USA	
RIKILT Institute for Food Safety, the Netherlands	Research into causes of food contamination with veterinary drug residues
Institute for Application of Atomic Energy, Department of Agro-Ecological Environment, Chinese Academy of Agricultural Sciences (CAAS), China	Development of methodology for food traceability and residues analysis

Institution	Topic
University of the Republic, Montevideo, Uruguay (UdelaR)	Method development and validation for pesticide residue control
International Federation for Animal Health (IFAH)	Quality control of trypanocidal drugs in sub-Saharan Africa
Global Alliance for Livestock Veterinary Medicines (GALVmed), Edinburgh, UK	
United Nations Office on Drugs and Crime (UNODC), Vienna, Austria	
Manchester Metropolitan University, UK	
Laboratoire de Contrôle des Médicaments Vétérinaires, Dakar, Senegal	
Tanzania Food and Drug Authority, Tanzania	
University of Otago, New Zealand	Collaboration on the use of stable isotope measurements for traceability of foods Development and validation of new certified reference materials for stable isotope analysis Research into new stable isotope techniques for verifying the integrity of honey products
Global Food Safety Partnership	Food safety capacity building
UNIDO	
International Food Safety Training Laboratory (IFSTL) of the University of Maryland/JIFSAN, MD, USA	
LVA GmbH (Lebensmittelversuchsanstalt - Food Research Institute) , Austria	Method development and optimisation for pesticide residue analysis Training developing country analytical chemists and technicians

THE INSECT PEST CONTROL LABORATORY

EXECUTIVE SUMMARY

In the livestock pest group of the Insect Pest Control Laboratory (IPCL), the impact of the endosymbiont *Wolbachia* on intersubspecies hybrid sterility was assessed, using two tsetse subspecies, *Glossina morsitans morsitans* (*Gmm*) and *Glossina morsitans centralis* (*Gmc*). A hybrid line was established by crossing *Gmm* females with *Gmc* males that had been treated with tetracycline to remove the endosymbionts. The mating compatibility of the hybrid flies derived from this colony was tested against both parent lines, *Gmm* and *Gmc*, and the result indicate no mating isolation of the hybrid flies. The data provides evidence for the involvement of *Wolbachia* in incipient speciation.

In tsetse species such as *Glossina pallidipes*, the presence of the salivary gland hypertrophy virus (SGHV) results in reproductive abnormalities. PCR analysis confirmed that the virus was not only present in *G. pallidipes* but also infects colonies of other tsetse species such as *G. m. morsitans*, however without any evidence of pathological symptoms. This implies that in *G. m. morsitans*, SGHV is either latent or undergoes limited replication without any disease symptoms. Specific host peptides might be involved in the expression or suppression of salivary gland hypertrophy (SGH) symptoms in *G. pallidipes* and *G. m. morsitans*, respectively. The identified proteins/peptides might be ideal candidates for the development of anti-virus control strategies in *G. pallidipes* colonies.

In the plant pest group, research was carried out on the remating behaviour of the Oriental fruit fly, *Bactrocera dorsalis*. Preliminary results showed that injection of accessory gland fluid from males (that had been fed with protein and methyl eugenol) to virgin females did not prevent copulation, while untreated females that mated with treated males where effectively inhibited from remating.

Efforts were undertaken to improve the genetic sexing strains (GSS) of the Mediterranean fruit fly, *Ceratitis capitata*, that carry a *white pupae* (*wp*) mutation and a *temperature sensitive lethal* (*tsl*) gene that allows the killing of colony females in the egg stage. Females that carry the *tsl* allele tend to develop slower, which is especially expressed during the larval phase. Experiments were carried out to attempt to remove the slow development trait from the *tsl* strain, and to isolate specific lines with a similar development time for female and male larvae. Fruit fly strains that have similar development times for male and female larvae have several advantages, such as males and females having access to the same quality of larval food, reduced space and energy consumption, and increased female larval survival and egg production.

The production and quality profile of different Mediterranean fruit fly GSS were assessed under semi mass-rearing conditions, i.e., the VIENNA-8 *tsl* strain where the females carry a homozygous viable D53 inversion, the VIENNA-8 *tsl* strain without the D53 inversion, and the Female Specific Embryo Lethality (FSEL-32) transgenic strain. Preliminary results indicate that the FSEL-32 transgenic strain had a similar production and quality profile as compared with the VIENNA-8 strains, irrespective of whether they carried the D53 inversion or not.

Bacteria belonging to the Enterobacteriaceae were assessed as potential additives to the larval diet as a probiotic (live bacteria) or as a nutritional supplement (dead bacteria). Preliminary results suggest that the use of dry, inactivated bacteria could be a viable alternative to the use of brewer's yeast for the rearing of the Mediterranean fruit fly.

In collaboration with the Institute de Recherche et de Développement en Agroenvironnement (IRDA) in Québec, Canada, research is underway to develop rearing methods for the spotted-wing drosophila (*Drosophila suzukii*) and develop radiation dose response curves and irradiation protocols.

Research continued under the FAO/IAEA/USDA agreement on "Development of Phytosanitary and Regulatory Treatments for Exotic Tephritid Fruit Flies" and has concentrated on cold treatments. The late 3rd instar larvae of *Bactrocera tau* (origin: China) and *Anastrepha grandis* were found to be the most cold-tolerant stage. Testing with various populations of *Bactrocera dorsalis* and *Ceratitis capitata* further indicates that the late 3rd instar larva is most tolerant to cold. This finding, which indicates that the 3rd instar is the most cold-tolerant stage across tephritid species, will facilitate development of broadly applicable cold treatments against Tephritidae, the most important pest group hampering trade in fresh fruits.

In the human disease vectors group, work was conducted on the possibility of reusing water to rear successive batches of mosquito larvae. Water can be collected when the larval rearing rack is tilted to collect pupae and used to rear the next round of mosquitoes. First results appear promising, with *Anopheles arabiensis* larvae developing well to adulthood in this reused water, though some impact was noticed on the quality of resulting adults.

Work was initiated on the development of aerial release systems for mosquitoes using unmanned aerial vehicles (UAV). The IPCL and collaborators (HEIGHT TECH of the Spectair group, Germany) are developing a prototype named Remotely Operated Mosquito Emission Operation (ROMEO), which combines an octocopter and a custom-built release device to deliver mosquitoes using an automated programme that is based on real time field surveillance data.

In the genetics and molecular biology group, work was continued on the development of GSS for mosquitoes. As a first step, we initiated the isolation of naturally occurring morphological and/or conditional lethal mutations and those induced through chemical mutagenesis (EMS-based mutagenesis screening) in three major mosquito vector species: *Anopheles arabiensis*, *Aedes albopictus* and *Aedes aegypti*. Candidate mutations have been detected in all species and, in all cases, they cause colour changes of the body of different development stages or of the eyes. Most of the mutations are recessive, some of them autosomal while others are sex-linked. The mutant strains established are currently being evaluated with respect to the potential impact of these mutations on the fitness and the biological quality of the insects.

Several laboratories and facilities have recently experienced difficulties in upscaling the production of the tsetse fly *Glossina fuscipes fuscipes*. The IPCL has initiated a detailed investigation of the microbiota associated with this tsetse fly species and the results currently available suggest the presence of a *Spiroplasma* species in both laboratory and natural populations of *G. f. fuscipes*. Our current research efforts focus on the impact of *Spiroplasma* on all aspects of the biology and ecology of this species in an attempt to improve rearing efficiency and enhance SIT applications.

STAFF

Name	Title
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Caceres, Carlos	Entomologist (Plant Pests)
Parker, Andrew	Entomologist (Livestock Pests)

Name	Title
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Adun, Henry	Laboratory Technician
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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Livestock Pests

Hybrid sterility between the tsetse fly subspecies Glossina morsitans morsitans *and* Glossina morsitans centralis *and the establishment of a stable hybrid line*

As mentioned in the Activities Report 2014, some tsetse subspecies (e.g. *Glossina morsitans morsitans* (*Gmm*) and *Glossina morsitans centralis* (*Gmc*)) can successfully mate with each other but they do not produce any offspring or, if offspring are produced, these are sterile. Cytoplasmic incompatibility (CI) leading to embryonic lethality has been shown to be caused by the endosymbiont *Wolbachia* Both *Gmm* and *Gmc* are known to harbour *Wolbachia*, and this might be the cause of the sterility in the F_1 resulting from crosses between the subspecies. We explored the impact of *Wolbachia* or any other tsetse associated bacteria on intersubspecies hybrid sterility by conducting a series of intersubspecies crosses using *Gmm* females mated to *Gmc* males treated with tetracycline (20µg/ml) to remove the symbionts. The results indicated that *Gmc* males that were offered six blood meals that had been treated with tetracycline before mating with untreated *Gmm* females produced a larger number of F_1 to F_7 offspring as compared with *Gmc* males that were only offered three blood meals supplemented with tetracycline. Further descendants from the *Gmc* males (fed six times on tetracycline supplemented blood) that had been crossed with *Gmm* females (fed with untreated blood) showed good performance in subsequent generations. This *Gmm* X *Gmc* hybrid colony is well established at the IPCL. The mating compatibility of the hybrid flies derived

from this colony was tested against both parent lines, *Gmm* and *Gmc*, and the result indicate no mating isolation of the hybrid flies. Although it is not conclusive, this is strong evidence for the involvement of *Wolbachia* in the production of CI and its involvement in incipient speciation.

Why is *Glossina pallidipes* more susceptible to salivary gland hypertrophy than *Glossina morsitans morsitans*?

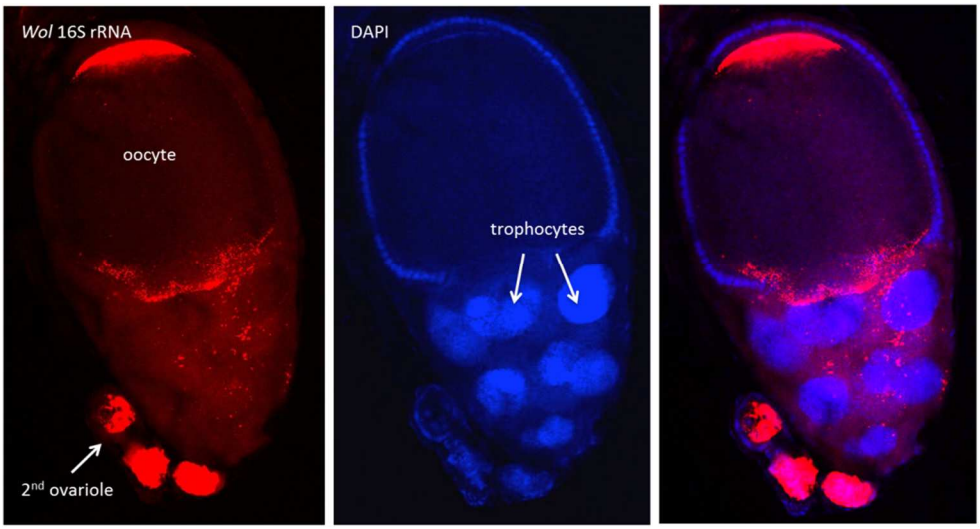

FIG. 1: DNA-RNA Fluorescent in situ hybridization (FISH) of Glossina morsitans morsitans *ovaries.* Wolbachia *(red) endosymbionts are concentrated at the posterior pole of the oocyte and around trophocytes (blue). The second ovariole is also heavily infected with* Wolbachia*.* Wolbachia *were stained with fluorescent-dye labelled oligoprobes targeting the symbiont 16S ribosomal RNA, host nuclei are stained with DAPI (Figure provided by Wolfgang Miller).*

As reported previously, some tsetse species carry a virus that in a certain individuals leads to salivary gland hypertrophy (SGH) and these individuals show reproductive abnormalities. In natural tsetse populations the SGH prevalence is in general low (0.5-5%). In a colony of *Glossina pallidipes* originating from Uganda and maintained at the IPCL, the frequency of SGH ranged from 4 to 10%.

The virus was also present in a *G. pallidipes* colony that was maintained at the Kality mass-rearing facility in Ethiopia, but the SGH prevalence was much higher and up to 77% of the flies were symptomatic. This high prevalence was the cause of the poor performance of the colony. PCR analysis confirmed that the virus was not only present in *G. pallidipes* but also infects colonies of other tsetse species such as *G. m. morsitans*, however, without any evidence of pathological symptoms. This seemed to indicate that *G. pallidipes* flies were more susceptible to the virus than *G. m. morsitans*

The differential sensitivity of *G. pallidipes* and *G. m. morsitans* colonies to the expression of SGH symptoms was assessed using qPCR and mass-spectrometry. Virus replication and total protein expression in the salivary glands of *G. pallidipes* and *G. m. morsitans* F_1 progeny was quantified and compared with their non-infected counterparts. These were the key findings: (i) whereas all the *G. pallidipes* F_1 progeny showed SGH symptoms, none of the *G. m. morsitans* F_1 progeny showed any detectable SGH symptoms (Fig. 1). This implies that in *G. m. morsitans*, SGHV is either latent or undergoes limited replication without any disease symptoms; (ii) 50% (9/18) of the proteins up-regulated in virus-infected *G. m. morsitans* were involved in the host's antiviral defence pathways, implying that this tsetse species is able to develop a more potent anti-virus defence than *G. pallidipes*; and (iii) approximately 40% (23/57) of the proteins up-regulated in virus-infected *G. pallidipes* were involved in at least ten different host signalling pathways, which together account for the development of SGH symptoms in *G. pallidipes*.

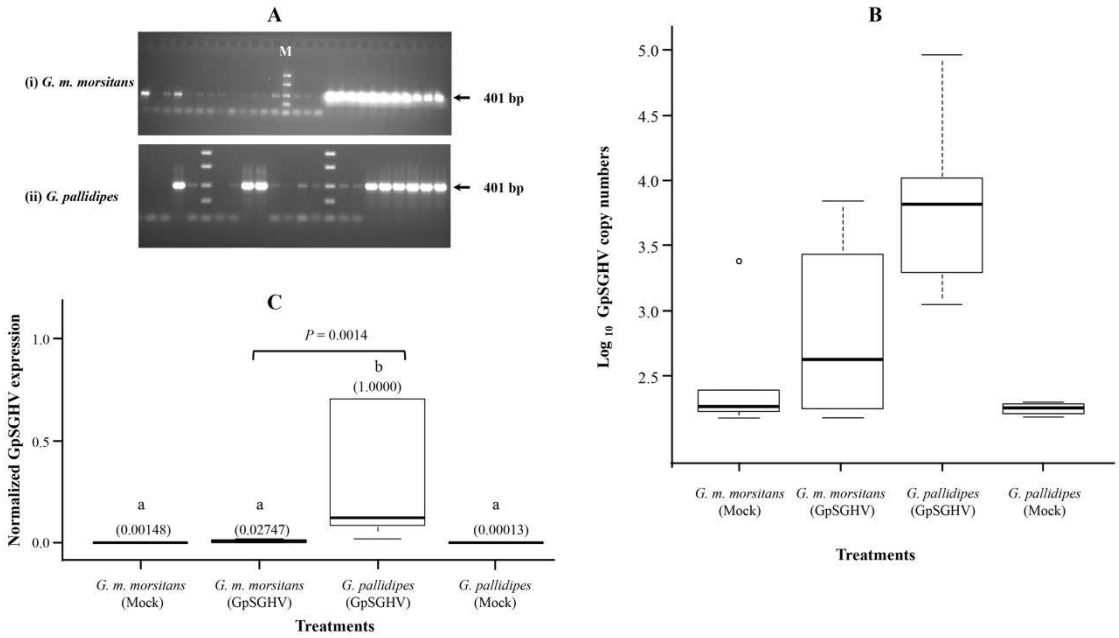

Fig. 2: Glossina pallidipes *salivary gland hypertrophy virus (GpSGH) replication in* G. m. morsitans *and* G. pallidipes*: (A) PCR-detection of SGHV infection in (i)* G. m. morsitans *and (ii)* G. pallidipes *F_1 progeny. qPCR quantification of: (B) GpSGHV titters and (C) GpSGHV expression levels. The GpSGHV expression levels (values shown in parentheses in Panel C) in G. pallidipes were significantly higher (P = 0.0014) than in* G. m. morsitans*. Letters a and b (in Panel C) represent significant differences between the samples (i.e. there was no significant difference between samples labelled a, while a and b were significantly different).*

These findings indicate involvement of specific host peptides (and their interactions with viral peptides) in the development or lack of overt SGH symptoms in *G. pallidipes* and *G. m. morsitans*, respectively. The identified proteins/peptides are ideal candidates for development of anti-virus control strategies in *G. pallidipes* colonies, e.g. by blocking virus replication, dissemination and development of SGH symptoms. This study has been published in the Journal *Frontiers in Microbiology*.

Plant Pests

Impact of chemotherapy, hormonal treatment and diet on the post-copulatory behaviour of Bactrocera dorsalis *and* Bactrocera cucurbitae

Post-teneral treatment of fruit fly males before release has proven to improve their sexual performance, i.e. addition of protein or plant oils to the adult food, topic application of juvenile hormone surrogates (e.g. methoprene), or feeding or exposure to methyl eugenol (ME) has been demonstrated to boost male sexual competitiveness. The combined effect of ME and protein increases the mating performance of male *Bactrocera dorsalis*. However, we lack a clear understanding on how post-teneral treatments such as ME can impact a male's ability to transfer sperm and inhibit females from remating. The efficiency of the SIT can be enhanced if wild females become refractory to remating with a wild male after mating with a sterile male. Mating inhibition is often achieved through the ejaculate, i.e., proteins produced in the male accessory glands and transferred to females during mating have important repercussions on female post-mating behaviour such as host location, oviposition and can act as anti-aphrodisiacs. Ms M. Reyes-Hernández of the Universidad Veracruzana, Xalapa, Veracruz, Mexico worked as a consultant at the IPCL to assess the effect of ME on the post-copulatory behaviour and ejaculate transfer of *B. dorsalis*. The remating behaviour of *B. dorsalis* females, which had previously been injected with accessory glands from males fed protein and ME, sugar and ME, only protein or only sugar, was assessed. In addition, she evaluated the effect of these post-teneral treatments on the size of the male accessory glands and the number of sperm transferred to females during copulation.

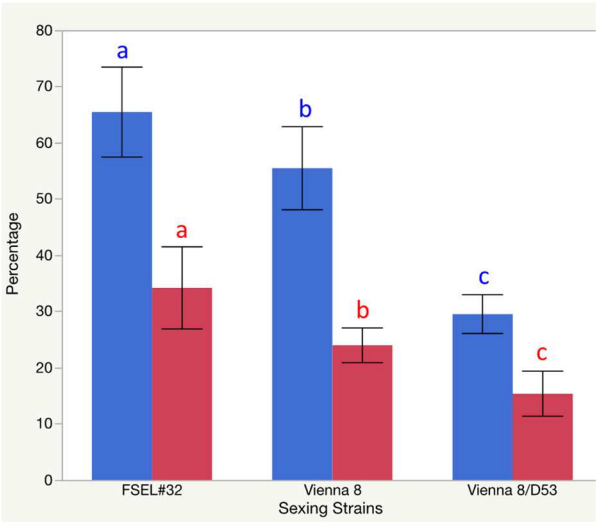

FIG. 3: Percentage recovery (egg to pupal development) of three strains of the Mediterranean fruit fly. Blue: total flies, brown: male flies

Preliminary results show that injection of accessory gland fluid from treated males to virgin females did not prevent copulation, whereas untreated females (those that were not injected with accessory gland fluids) that mated with treated males were effectively inhibited from remating. Analysis on the amount of sperm transferred and the size of the accessory glands are ongoing.

Improvement of the temperature sensitive lethal *(tsl) Genetic Sexing Strain of the Mediterranean fruit fly*

Females of the Mediterranean fruit fly *Ceratitis capitata temperature sensitive lethal* (*tsl*) strain carry two markers that are used to separate male and female flies, i.e. a *white pupae* (*wp*) mutation that allows the separation of male and female pupae by colour and a *tsl* gene that allows the killing of colony females in the egg stage. Both alleles are located close to each other on the right arm of autosome 5. Females that carry the *tsl* allele tend to develop slower, which is especially expressed during the larval phase with female larvae requiring three additional days to complete larval development in comparison with male larvae. This characteristic itself can be used to facilitate the separation of the sexes in mass-raring facilities as early larval collections are mostly males, whereas late larval collections are mostly females.

At the El Pino Mediterranean fruit fly mass-rearing facility in Guatemala, it was observed that pupal trays of early larval collection days contained only few white pupae (females), whereas most pupae were brown (male pupae), i.e. 1 white for 100,000 brown pupae. White pupae from this early larval collection were taken to the quarantine station of the Petapa Mediterranean fruit fly facility to determine whether the females that emerge from these white pupae still carry the allele responsible for the sensitivity to temperature. The females were still sensitive to high temperatures and it was concluded that the slow development trait of the larvae of the *tsl* strain was covered by a different allele, which was lost by the white *tsl* pupae females due to normal genetic recombination.

At the IPCL, experiments were carried out to attempt to remove the slow development trait from the *tsl* strain of the Mediterranean fruit fly, and to isolate specific lines that have a similar development time for the female and male larvae. From a total of 200 families, eleven lines were isolated and one (line 27) was retained for further analyses.

The following are the advantages of rearing fruit fly strains that have similar development times for male and female larvae:

(a) Males and females will have access to same quality of larval food.
(b) Mass-rearing facilities will be able to reduce space and energy consumption for holding the colony as the total time for larval development will be reduced.
(c) Preliminary experiments have shown that removing the slow development trait from the *tsl* strain will increase female larval survival and egg production.
(d) Attributes (b) and (c) will substantially reduce the rearing cost of the *tsl* strain and will therefore make the SIT more cost efficient.

Large-scale experiments will be required to determine the biological profile of the new strain that has been named VIENNA-9 before the strain can be transferred to action programmes.

The mass-rearing profile and quality characteristics of GSS of the Mediterranean fruit fly constructed using transgenic or classical genetics approaches

Colleagues of the Department of Developmental Biology, Georg-August-University Göttingen, Germany have developed a transgenic GSS of the Mediterranean fruit fly, *Ceratitis capitata* that is based on female-specific embryonic lethality. In this transgenic strain, female lethality is expressed at the embryonic stage by depriving adults of tetracycline. Colonies that are provided with tetracycline produce equal proportion of males and females. This strain shows promise in view that it eliminates the female flies at an early development stage. It will, however, have to compete with the standard GSS of the Mediterranean fruit fly that were developed using classical genetic methods and that are based on temperature sensitive lethality (and that are now used in most Mediterranean fruit fly mass-rearing facilities in the world). However, this transgenic approach could be easily transferred to other fruit flies of economic importance or disease vectors.

The IPCL hosted a visiting scientist from Mexico, Mr Salvador Meza (Moscafrut/Moscamed) who compared, at a semi-large rearing level, the production and quality profile of different Mediterranean fruit fly strains, i.e., the VIENNA-8 *temperature sensitive lethal* strain where the females carried a homozygous viable D53 inversion, the VIENNA-8 *temperature sensitive lethal* strain without the D53 inversion, and the Female Specific Embryo Lethality (FSEL-32) transgenic strain. Preliminary results indicate that the FSEL-32 strain had a similar production and quality profile as compared with the VIENNA-8 GSS irrespective of whether they carried the D53 inversion or not (Fig. 3). Although there are regulatory hurdles that need to be overcome with the use of transgenic strains in operational programmes against the Mediterranean fruit fly, the data of this evaluation show that transgenic genetic sexing approaches might be worth considering for other insect species in programmes that have an SIT component.

Use of bacteria as a source of protein and essential amino acids in the larval diet of the Mediterranean fruit fly

The Genetics and Molecular Biology group of the IPCL has been isolating gut associated bacteria from the Mediterranean fruit fly. One of these bacteria belongs to the Enterobacteriaceae and previous experiments showed that adding these to the larval diet as a probiotic (live bacteria) or as a nutritional supplement (dead bacteria) improved productivity. In collaboration with colleagues from the Democritus University of Thrace, Greece, small bioreactors were used to produce these bacteria in sufficient numbers for experiments to assess whether they could be used to replace brewer's yeast as a source of protein and essential amino acids in the larval diet of the Mediterranean fruit fly. Preliminary results suggest that the use of dry, inactivated bacteria could be a viable alternative to the use of brewer's yeast for the rearing of the Mediterranean fruit fly. More research is needed to confirm our preliminary results and a benefit-cost analysis needs to be made to assess the economic viability of this approach.

Drosophila suzukii

In collaboration with the Institute de Recherche et de Développement en Agroenvironnement (IRDA) in Québec, Canada, research is underway to develop rearing methods for the spotted wing drosophila (*Drosophila suzukii*) and to develop radiation dose response curves and irradiation protocols. This research is part of an assessment of whether the SIT could potentially be integrated with other control tactics in greenhouses or other confined areas against this invasive pest.

Anastrepha fraterculus *complex*

The IPCL hosted three visiting scientists from Argentina (Ms María Laura Juárez, Mr Fransico Devescovi and Mr Diego Segura) in the second half of 2015. They further explored the role of the male sex pheromone of the different morphotypes of *Anastrepha fraterculus*. Experiments in field cages were carried out to 1) determine the females' responses to pheromones of males from their

own morphotype or other morphotypes using artificial leks; 2) determine the hetero-specific mate recognition system by assessing long and short distance attraction between males and females from the different morphotypes and/or populations; 3) describe the temporal pattern of calling and mating behaviour for each morphotype/population. In addition, volatiles from calling males of the different populations were collected for further bioassays.

Populations from Castelar (Argentina), Vacaria and Piracicaba (Brazil) that belong to the Brazilian-1 morphotype, and one population from Mexico that belongs to the Mexican morphotype, were used for the different experiments. Collections of volatiles from these populations and one from Peru have been carried out. Data analyses of all experiments are in progress.

Phytosanitary treatments under the FAO/IAEA/USDA agreement

Research under the FAO/IAEA/USDA agreement, "Development of Phytosanitary and Regulatory Treatments for Exotic Tephritid Fruit Flies" has concentrated on cold treatments in 2015 with the objective of taking advantage of the tephritid resources at the IPCL to develop broadly applicable phytosanitary cold treatments. The results of this research will guide phytosanitary treatment scheduling at the national and international levels among FAO and IAEA Member States as well as at the International Plant Protection Convention (IPPC). Collaboration with the IPPC through the Technical Panel on Phytosanitary Treatments and the IPPC-liaison organization, the Phytosanitary Measures Research Group, leverages cooperative international efforts toward the development of broadly applicable phytosanitary treatments.

Cold treatment research is being conducted with two species for which phytosanitary research in any category has not yet been done, *Bactrocera tau* and *Anastrepha grandis*. *Bactrocera tau* was obtained from China and the late 3rd instar was found to be the most cold-tolerant stage. Large-scale testing is currently underway to confirm a phytosanitary treatment dose (time) at 1°C that would serve as a commercial treatment against this species. Rearing techniques were developed for *A. grandis*, and the most cold-tolerant stage was also identified as the late 3rd instar. Testing with various populations of *Bactrocera dorsalis* and *Ceratitis capitata* further indicates that the late 3rd instar is most tolerant to cold. This finding, which indicates that the 3rd instar is the most cold-tolerant stage across tephritid species, will facilitate development of broadly applicable cold treatments against Tephritidae, the most important pest group hampering trade in fresh fruits.

Populations of *C. capitata* from Argentina, Australia, and Spain are being compared to determine if they differ in cold tolerance. Similar research is being conducted with *B. dorsalis*, and if populations of the same species are not found to differ in cold tolerance it will facilitate the development of broadly applicable cold treatments.

Hot water immersion treatment research on mangoes infested with *B. dorsalis* was conducted at the IPCL with a visiting scientist from Mozambique to help develop a hot water immersion treatment that would allow for export of mangoes to South Africa and other markets lost when *B. dorsalis* invaded Africa. That work is continuing in Mozambique.

Manuscripts supporting several generic phytosanitary irradiation treatments arising from a recently concluded Coordinated Research Project on generic phytosanitary irradiation doses have been accepted for publication. These will be submitted to the IPPC and national plant protection organizations for consideration as commercial treatments to disinfest commodities in international trade of regulated pests.

Human Disease Vectors

With mass-rearing protocols optimised and standardised, and surveillance activities well advanced in several Member States, the work of the Human Disease Vectors group of the IPCL is advancing to support several pilot suppression trials, developing release and trapping methods alongside up-scaled mosquito production.

FIG. 4: Mr Mamai Wadaka of Cameroun, operating a rack system for mosquitoes. Rearing larvae of mosquitoes in larval trays stacked in a mass-rearing rack system requires large quantities of water, but the environmental footprint could be reduced if the water could be recycled.

Reusing larval rearing water to minimise the environmental and economic cost of mosquito production

Mosquitoes are reared efficiently on a large scale using the mass-rearing equipment designed and tested at the IPCL, as reported previously, with 200,000 *Anopheles arabiensis* eggs, for example, being cultured in one larval rearing rack (Fig. 4). Around 250 litres of water is required per rack, making the availability of clean water and disposal of waste water key considerations in the running of a mosquito production facility. Many countries in which mosquitoes are endemic are located in arid zones where water provision can be costly or unreliable; it would be beneficial to the sustainability of mosquito production if water use could be reduced.

The possibility of reusing water to rear successive batches of mosquito larvae is therefore being investigated. Water can be collected when the larval rearing rack is tilted to collect pupae and used to rear the next round of mosquitoes. Results are promising, with *Anopheles arabiensis* larvae developing well to adulthood in this reused water, though some impact was noticed on the quality of resulting adults. Further investigations are therefore ongoing into water treatment processes that might render the water reusable without any detrimental effect on the mosquitoes produced. It is hoped that a simple method can be developed for treating large quantities of water for reuse, which would be cost effective in reducing the water requirements and subsequent running costs of a mosquito mass rearing facility, making the SIT a more feasible approach to mosquito control.

Unmanned Aerial Vehicles (UAV) for aerial release of adult mosquitoes

For an effective mosquito population suppression programme, sterile male mosquitoes must be released (or deployed) over the target area at a fine enough scale to give good coverage, even considering the relatively short distances over which they will disperse. Often target areas are difficult to cover with ground vehicles, being remote or very rural and lacking in roads that are passable year round. Aerial release is therefore an attractive alternative, and given the small size and low weight of adult mosquitoes a large area could potentially be treated with sterile males using a suitable unmanned aerial vehicle (UAV). While payload and flight time must be balanced, a variety of UAVs with the right

The ROMEO team: IPCL staff and HEIGHT TECH (Spectair Group) engineering experts develop an unmanned aerial vehicle and associated release device for deployment of sterile male mosquitoes by air

characteristics are commercially available and the software for automated release of a spatially prescribed number or density of insects is already in use in SIT programmes against other species. A release device must be developed specially for mosquitoes, and the complete system optimised for release of *Aedes* and *Anopheles* adults.

The IPCL and collaborators (HEIGHT TECH of the Spectair group, Germany) are developing a prototype named "Remotely Operated Mosquito Emission Operation" (ROMEO), which combines an octocopter and a custom-built release device to deliver mosquitoes in an automated programme designed in advance based on real time field surveillance data. To load adult mosquitoes into the release device they must be immobilised by chilling; ideally adults will be chilled in the mass-rearing facility directly into the release device for transport to the release site and loading into the UAV. The effect of low temperature for prolonged periods on adult survival and subsequent performance, and development of transport and chilling devices and protocols are therefore being investigated and developed as part of a Coordinated Research Project on 'Mosquito handling, transport, release and male trapping methods which was launched in 2015.

Genetics and Molecular Biology

Isolation of mutations for the development of mosquito genetic sexing strains for SIT applications

There is an increasing demand by Member States to develop and apply the SIT to control populations of mosquito disease vector species transmitting major human pathogens causing diseases such as malaria, dengue, chikungunya, zika, yellow fever and others. A critical factor for the development and application of the SIT package for the management of mosquito populations is sex separation. It's absolutely necessary for the mosquito SIT applications to be based entirely on sterile male-only releases because females are the transmitting sex of the major human pathogens mentioned above. There is therefore an urgent need to develop robust and efficient genetic sexing strains (GSS) which can absolutely ensure male-only releases. The construction of a classical GSS requires two main components: (a) a selectable marker, which is necessary for sex separation or female-killing and (b) a translocation, which is required to link the inheritance of this marker to sex. In *Anopheles* species this should be a Y-autosome translocation while in mosquitoes with homomorphic sex chromosomes, like *Aedes* species, the translocation must be to the male-determining factor. As a first step towards the construction of stable and efficient GSS, we initiated the isolation of naturally occurring morphological and/or conditional lethal mutations (like the temperature sensitive lethal mutation) and those induced through chemical mutagenesis (EMS-based mutagenesis screening) in three major mosquito vector species: *Anopheles arabiensis*, *Aedes albopictus* and *Aedes aegypti*. Candidate mutations have been detected in all species and, in all cases, they induce colour changes of the body at different development stages or of the eyes. Most of the mutations are recessive, some of them autosomal while others are sex-linked. The mutant strains established are currently being evaluated with respect to the potential impact of these mutations on the fitness and the biological quality of the insects. The best strains will be used in irradiation experiments in order to induce translocations and link the inheritance of these markers to sex. This will eventually result in the construction of GSS that could be used in SIT applications to control populations of these major mosquito vector species worldwide.

Tsetse flies and Spiroplasma *infection*

Tsetse flies are the cyclical vectors of African trypanosomes, which cause sleeping sickness in humans and a similar disease called nagana in animals. As yet, there are no effective vaccines available and the treatment of trypanosomosis is often based on the use of noxious, difficult to administer, and expensive drugs. Trapping- and pesticide-based management are available; however, they do not offer efficient or sustainable solutions. The sterile insect technique (SIT) has shown potential as an environment-friendly method to manage the disease through the control of

tsetse populations. One of the most critical factors to implement the SIT for tsetse flies is the mass-rearing of the target insect species due to its unique reproductive biology (tsetse flies produce one progeny every 9-10 days) and their sensitivity to parasitic infections. There have been reports on the collapse of tsetse colonies (e.g. *Glossina pallidipes*) in both laboratory and mass-rearing facilities, some of which had been due to the presence of the salivary gland hypertrophy virus (SGHV). Several laboratories and facilities have recently experienced difficulties in upscaling the production of *Glossina fuscipes fuscipes* in the frame of ongoing efforts to control this species through SIT applications. To identify potential causal factors, we have initiated a detailed investigation of the microbiota associated with this tsetse fly species in collaboration with Prof. George Tsiamis of the Department of Environmental and Natural Resources Management, University of Patras, Greece. Different molecular and microscopy methods (PCR screening methods, 16S rRNA gene sequencing, qPCR, FISH) have been used to characterize the associated microbiota. The results currently available suggest the presence of a *Spiroplasma* species in both laboratory and natural populations of *G. f. fuscipes*. *Spiroplasma* is a group of wall-less bacteria that have been detected in diverse plants and arthropods. These bacteria can live both intracellularly and extracellularly. In addition, *Spiroplasma* has been characterized as symbionts providing protection in some insect species against nematodes, wasps and fungi but they have also been reported as pathogenic in other insects, crustaceans and in plants. In addition, *Spiroplasma* strains have been shown to induce male-killing phenomena in some insects. Our initial results suggest that *Spiroplasma* infection is more prevalent in the *G. f. fuscipes* colony maintained at the Kality facility in Ethiopia, as compared with the colony maintained at the IPCL, despite the fact that both colonies are of the same origin. Taking into consideration the widespread occurrence of this bacterial species in *G. f. fuscipes*, our current research efforts focus on the impact of *Spiroplasma* on all aspects of the biology and ecology of this species in an attempt to improve rearing efficiency and enhance SIT applications.

CAPACITY BUILDING AND SERVICES

In 2015, the IPCL hosted four cost-free experts (CFE), 11 consultants (C), 16 interns, eight fellows and three scientific visitors (SV) (the latter two categories funded by the IAEA's Department of Technical Cooperation) in the following areas:

Name	Country	Status	Duration	Topic
ABDELAZIZ ABBA, Ramadan	Egypt	Intern	8 mth	Hybridization of tsetse
KYRITSIS, Georgios	Greece	C	12 mth	Wolbachia in Mediterranean fruit fly
MORAN, Zelda	USA	Intern	6 mth	Pupae separation tsetse
RAS, Erica	Netherlands	Intern	6 mth	Probiotics olive fly
KALANTAROW, Inessa	Israel	Intern	12 mth	Endosymbionts tsetse flies
HALLMAN, Guy	USA	CFE	12 mth	Post harvest treatment of fruit flies
DEMIRBAS, Guler Uzel	Turkey	C	12 mth	Endosymbionts tsetse flies
MEKI, Irene	Kenya	Intern	12 mth	Virus control tsetse

Name	Country	Status	Duration	Topic
LANOUETTE, Genevieve	Canada	Intern	6 mth	Rearing Drosophila suzukii
AGUIR MASET, Bruno	Brazil	Intern	5 mth	Post harvest treatment of fruit flies
BRINOVEC, Masa	Slovenia	Intern	2 mth	Rearing Drosophila suzukii
BRUNET, Robin	UK	Intern	5 mth	Rearing mosquitoes
LUTTERI, Alexa	Italy	Intern	6 mth	Rearing Drosophila suzukii
WEILER, Dorottya	Hungary	Intern	11 mth	Reference manager
CARAVANTEs, Silvana	Guatemala	Intern	2 wk	Drosophila suzukii
JUAREZ JOSE, Guillermo	Guatemala	Intern	6 wk	Rearing mosquitoes
PROTOLIPAC, Katharina	Hungary	Intern	1.5 mth	Pupae separation tsetse
SEGURA, Diego	Argentina	CFE	3 wk	Mating studies fruit flies
JUAREZ, Maria Laura	Argentina	CFE	3mth	Mating studies fruit flies
DEVESCOVI, Francisco	Argentina	C	4.5 mth	Wolbachia in fruit flies
LEES, Rosemary	UK	C	12 mth	Mosquitoes
MAIGA, Hamadou	Burkina Faso	C	6.5 mth	Rearing mosquitoes
MEZA, Salvadore	Mexico	C	6 mth	Genetic sexing strains fruit fly
PASSOS RORIZ, Kelly	Brazil	C	2 mth	Fruit fly rearing
TARET, Gustavo	Argentina	C	1.5 mth	Rearing Drosophila suzukii
WADAKA, Mamai	Cameroun	C	12 mth	Mosquito rearing
CARVAHLO, Danilo	Brazil	C	1 mth	Mosquito genetics
BOND, Juan Guillermo	Mexico	C	1mth	Mosquito rearing
CULBERT, Nicole	UK	Intern	12 mth	Mosquito rearing
ZHANG, Dongjing	China	CFE	6 mth	Mosquito rearing
BIMBILE, Severin	Burkina Faso	Intern	2 mth	Mosquito rearing
AYAD AHMED, Al-Taweel	Iraq	SV	2 wk	Screwworm

Name	Country	Status	Duration	Topic
NIRAGIRE, Ildephonse	Rwanda	SV	2 d	Tsetse
PATEL, Nausheen Azhaar	Mauritius	SV	1 wk	Mosquitoes
WOOD, Oliver Richard	South Africa	Fellow	2 wk	Mosquitoes
KAISER, Maria	South Africa	Fellow	2 mth	Mosquitoes
MUOSA ALI, Zaynab Ibrahim	Sudan	Fellow	3 mth	Mosquitoes
KHAN, Gul Zamin	Pakistan	Fellow	6 mth	Mosquitoes
ROBERTSON, Leanne Nicole	South Africa	Fellow	3 mth	Mosquitoes
NOMAN, Keder	Sudan	Fellow	3 mth	Mosquitoes
NYENDA, Stanley	Zimbabwe	Fellow	2 wk	Tsetse
CHIGIYA, Tendai	Zimbabwe	Fellow	2 wk	Tsetse

In 2015, the plant pests group delivered 41 shipments (~125 000 pupae) of different fruit fly species to 14 research institutes in Argentina, Australia, Cameroun, Chile, Czech Republic, France, Germany, Greece, Israel, Italy, Mauritius, Mexico, Morocco, South Africa, Spain, Sweden and Switzerland. The livestock pests group had 42 shipments (~11 500 tsetse pupae) to six research institutes in France, Germany, Greece, Italy, Switzerland and United Kingdom. The human disease vector group had nine shipments of mosquito eggs (~52 000) to Germany, Italy, Sweden and Switzerland.

The IPCL delivered 34 shipments of preserved fly samples to 18 institutions in Argentina, Burkina Faso, Czech Republic, Denmark, France, Germany, Greece, Japan, Korea, La Reunion, Pakistan, South Africa, Tanzania, Thailand, Tunisia, Uganda, United Kingdom and USA; and had eight shipments of larval diet to seven institutions in Germany, Guatemala, Italy, Mauritius, Sri Lanka, Sudan and USA.

PUBLICATIONS

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THE PLANT BREEDING AND GENETICS LABORATORY

EXECUTIVE SUMMARY

The Plant Breeding and Genetics Laboratory (PBGL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture focusses on mutation breeding to increase biodiversity for desired traits of crop plants and hence to accelerate the breeding of varieties with higher yield, yield stability, nutrition and improved resistance to environmental stresses such as disease, drought and salinity. It plays a key role in the implementation of the Plant Breeding and Genetics subprogramme and provides assistance to Member States in the fields of mutation induction, mutation screening and mutation discovery.

PBGL continued its research activities towards finding alternatives to gamma irradiation, whose use in many countries is affected by restrictions imposed on the transfer of radioactive materials and difficulties in establishing new gamma irradiators, or refitting old ones. The experiments on mutation induction using X-ray irradiation have been further developed for both seeds and vegetatively propagated crops. These include analysis of the effect of X-rays on fertility and seed setting of the M1 generation, visual scoring of M2/M3 generations in field experiments and expanding of tests for determining optimal irradiation doses for crops such as diploid and tetraploid mustard, japonica and indica rice, soybean, tomato, chilli, durum wheat and sorghum. Based on the results a comprehensive protocol on X-ray mutagenesis is being drafted, which will provide breeders with the details on the technology as well as serve as a decision tool in the identification of the most suitable method for mutation induction. The protocol will be published in 2016.

With regard to the use of ion beams as a further alternative to gamma rays for mutation induction, different dose ranges have been tested and additional crop species have been explored. The experiments were facilitated through the existing collaboration with the Ruđer Bošković Institute in Zagreb, Croatia. Whereas in previously tested crops a similar trend of growth reduction with increased doses was detected between X- or gamma ray irradiation on the one hand and ion beam irradiation on the other hand, it was now shown that this is not true for all crops; for example in sorghum there was no reduction in seedling growth within the previously applied dose range. This indicates the need for extensive radiosensitivity tests before carrying out bulk irradiation. This is considered especially important since ion beams may indeed induce different types of mutations as compared to gamma rays or chemical mutagens. It is for example possible that ion beams may induce mutations in the low dose ranges that do not greatly affect plant survival. Molecular studies, including those using whole genome sequencing techniques and detection of activated mobile genetic elements, would help to fully explore the potential of ion beam, as well as gamma and X-ray irradiation for crop improvement. PBGL will continue its efforts on the optimization of ion beam irradiation for mutation induction in order to generate protocols and provide practical guidelines to the plant breeders in Member States.

Mutation discovery based on the evaluation of DNA and linkage of the determined changes to improved characters provides breeders with invaluable tools for incorporating mutant lines and varieties into their breeding programmes. In this context the PBGL has further advanced the techniques and protocols for low costs DNA methods. Invitations to teach these techniques in international institutes demonstrated the interest in the methods that produce high quality DNA suitable for mutation discovery experiments. In order to be able to detect the rare and larger genomic aberrations, the kind of mutations characteristic for gamma irradiation, the PBGL is developing methods for whole genome analysis for the recovery of such mutations. Preliminary analysis of data from 17 sequenced mutant rice lines suggests that mutations induced by gamma irradiation can be effectively recovered from rice. The PBGL will use these data in developing a guideline for marker development.

In 2015 the PBGL was successful in attracting a grant as co-investigator of a project on the identification of natural mutations of genes in cassava that play a role e.g. in starch quality. This project is implemented together with the International Center for Tropical Agriculture (CIAT) in Colombia and financed by Colciencias, the Colombian governmental research foundation. In addition to acquiring more information on genetic variation in cassava, this project will greatly contribute to the development of methods for processing thousands of DNA samples for use in high throughput techniques for mutation discovery.

Training remains one of the main pillars of the PBGL's activities. Fifteen fellows from 11 Member States stayed in the lab for two to seven months and in some cases brought their own plant material for mutation induction, such as local cassava genotypes from Sierra Leone and the Central African Republic. In addition, the PBGL hosted ten Scientific Visitors from ten Member States and six Interns from five Members Sates. A two-week regional training course on 'Phenotyping and Genotyping of Mutants for Abiotic Stresses in Cereals' made up a part of the PBGL training programme, as did a series of short ad-hoc training courses on specific topics.

The irradiation service at PBGL continued to be a sought-after activity; in 2015 a total of 30 requests for plant irradiation services were received from 20 Member States covering 27 different crop species. This included both bulk irradiation and radiosensitivity testing.

In 2015 the PBGL staff were directly involved in 23 scientific publications, thereof seven original or review papers, two book chapters, one book and 13 abstracts presented at international conferences. One of the highlights was the publication by Springer of the protocol book on 'Low-Cost Methods for Molecular Characterization of Mutant Plants', which is available for free from the publisher's website.

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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

The Plant Breeding and Genetics Laboratory (PBGL) aims to support plant scientists engaged in crop improvement through the use of induced mutations. Our activities reside within the Plant Breeding and Genetics (PBG) subprogramme of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. They fall under three main categories: research and development to improve plant mutation breeding, capacity building for individuals and groups by disseminating expertise and methods developed/adapted at the PBGL, and the provision of plant irradiation services. Research and development activities focus on improving the efficiency of mutation induction, mutant plant screening and the development of methods that can easily be transferred to laboratories with limited infrastructure through the reduction in assay costs and toxic chemicals.

Mutation induction

The PBGL continued its ongoing activities to produce comprehensive protocols on the use of existing X-ray resources for mutation induction and exploring the potential of ion beam irradiation for efficient and wider spectrum mutagenesis.

Mutation induction using X-ray irradiation

In 2015 PBGL successfully concluded comprehensive experiments on the use of X-ray irradiation for mutation induction in seeds and vegetatively propagated crop plants. This included: 1) analysing the effects of X-ray irradiation on fertility and seed setting of the M1 generation, 2) extending the radio-sensitivity test to determine the optimum dose of mutation induction treatment in other seed and vegetatively propagated crop plants and 3) assessing the mutation rate based on visual scoring of mutant phenotypes in the M2/M3 generations both in the experimental field of the PBGL and in collaboration with Member States in representative crops, such as wheat and sorghum in Sudan and tomato in Senegal.

In addition to previously tested crops, such as bread wheat, barley, chilli, sesame and sunflower, the X-ray mutation induction procedure was further optimized for diploid and tetraploid mustard, japonica and indica rice, soybean, tomato, chilli, durum wheat and sorghum using the in-house RS 2400 Rad X-ray irradiator with a highest dose rate of around 10 Gy/min. Radio-sensitivity was scored at germination, early seedling growth and seed setting stages in the glasshouse and the field (Fig 1). The optimal dose of X-ray irradiation based on 50% and 30% reduction in germination and/or growth was comparable to gamma-ray in most of the tested crops. Seed setting decreased with the increase of absorbed dose from above 90% in the untreated control to around 20-30% with the increase in the dose to 300-450 Gy, while in some crop species no seeds were produced in surviving plants that

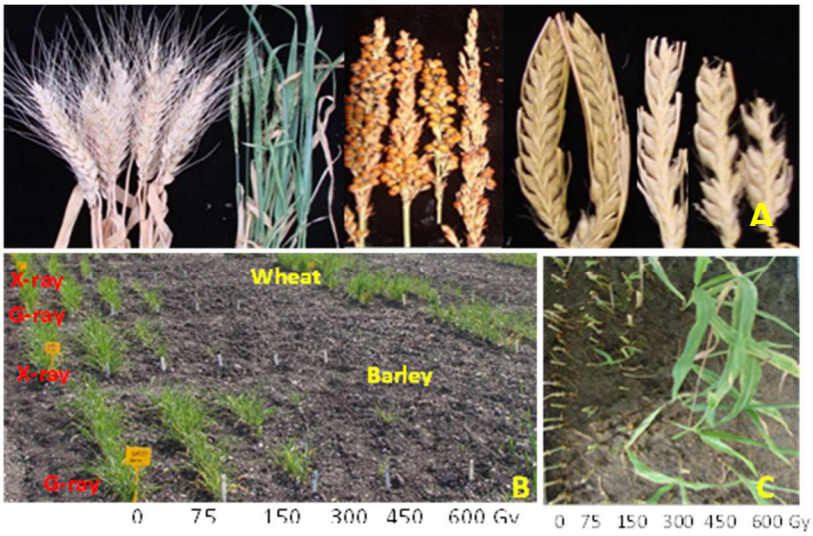

FIG. 1: A: Seed setting in heads of M1 plants of wheat, sorghum and barley produced from seeds treated with 0, 75, 150, 300, 450 and 600 Gy (ordered from left to right) of X-ray; B: Part of the PBGL field showing a radio sensitivity experiment comparing X-ray and gamma-ray irradiation; C: Photo taken after harvesting sorghum M1 plants treated with different doses of X-ray showing that most of the plants with high dose treatment failed to produce seeds

received more than 300 Gy when the M1 was planted in the glasshouse (Fig. 1C). Generally, the in-house X-ray machine produced slightly stronger effects on germination, early seedling growth and seed setting in M1 plants than the in-house ^{60}Co gamma source (Gammacell 220) with a dose rate of about 140 Gy/min (Fig. 1). Mustard seeds showed exceptional tolerance to irradiation and an effect on growth of M1 plants could be observed only when the treatment dose was increased to 1500 Gy in the diploid *Brassica nigra*; in the tetraploid *B. napus* slight temporal differences were observed in plant growth only at high dose treatment (600 Gy). This response was similar to that observed previously with gamma rays. This may suggest that for some highly irradiation insensitive seed crops, like mustard, the conventional radiosensitivity test based on germination and growth rate might not be suitable to determine the optimum dose of irradiation for mutation induction. Molecular discovery of mutations based on genome sequencing may shed light on the actual mutation frequency. In addition, enhancing seed moisture before treatment may be a means to increase the mutagenic effect of irradiation within a feasible dosage range.

Generally, the mutation frequency is comparable for both X-ray and gamma ray irradiation. For example the percentage of mutant plants in 10,000 M2 population of wheat ranged between 0.07 to 0.19% and 0.08 to 0.23% for X-ray and gamma-ray, respectively. Fig 2 shows representative photos of mutants scored in M2/M3 populations of tomato and chilli in the field of the PBGL.

FIG. 2: Representative phenotypes of X-ray generated M2/M3 in the experimental field of PBGL showing variation in maturity (A vs B), fruit shape(C), leaf shape and colour (D), and plant size and shape in tomato (A-C) and chilli (D)

These experiments show that X-ray treatment is a comparable alternative to gamma irradiation for mutation induction; however, radiosensitivity tests need to be carried out on each species and variety before implementing such treatment. To facilitate this we plan to include a comprehensive reference table of major seed crops in the planned book of '*Protocols for mutation induction using X-ray irradiation in seed and vegetatively propagated crops*' to assist Member States in the optimization process of their mutation induction activities.

Optimization of ion beam irradiation for mutation induction in crops

The optimization of ion beam irradiation for mutation induction was initiated in 2014. In 2015 the focus was on determining the optimal ion beam dose range for different crop varieties. After moisture equilibration to 12-14%, seeds of representative crops were irradiated at the Ruđer Bošković Institute in Zagreb, Croatia, to 0, 40, 70, 140, 180, 240 and 340 pA (Fig. 3). The crops included cereals (sorghum, rice, wheat and barley), oil crops (sesame, *B. napus* and peanut) legumes (bean and lentil) and horticultural crops (tomato and chilli). Treated M1 seeds were planted at the PBGL glasshouse, and treatments were compared in parallel with samples irradiated with the in-house X-ray and gamma ray sources. While the radiosensitivity to X-ray and gamma ray followed a

similar trend of growth reduction with increasing dose rate, the situation was different with the ion beam applied treatments among different crop species (Fig. 3, C and D).

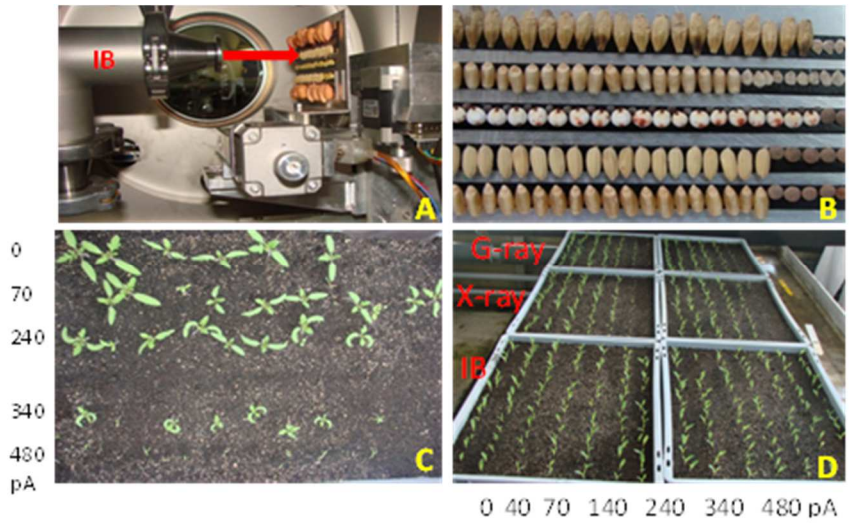

FIG. 3. Platform of seed samples exposed to beam irradiation (A). Seeds of different crop plants mounted on aluminium platform ready for irradiation (B), radiosensitivity of M1 seedling of tomato (C) and comparison of sensitivity of M1 sorghum among ion beam (IB), X-ray and Gamma-ray doses of 0 to 600 Gy from left to right (D)

While in some crops, like tomato, the trend of reduction in seedling growth with increasing dose was maintained (Fig. 3C), the difference was not distinguishable in most of the other treated crop plants, including sorghum (Fig. 3D). Seeds of the M1 were harvested and will be planted and evaluated for mutation rates in M2 progenies. Further dose optimization experiments will need to be conducted to determine comparable trends in radio-sensitivity using X-ray and gamma ray.

Mutation Screening and Mutation Discovery

Screening for salt tolerance in soybean using hydroponic culture

In the context of climate change, there is great challenge to maintain sustainable food production to feed a growing world population. The PBGL has been involved actively in developing efficient and reliable screening methods for the development of salt tolerant mutant varieties. Screening packages against salinity stress are being designed and evaluated to suit different crops under different conditions in the laboratory, glasshouse and open field. During the reporting period an experiment was conducted for salinity screening in soybean genotypes from Bangladesh.

Twenty soybean genotypes were used for salinity screening using hydroponic culture. Sodium chloride (NaCl) was used at 0, 5, 10 and 15 dS/m concentrations in a hydroponic system with modified Ishida's nutrient solution. Salinity stress was imposed at the early vegetative stage (nine days after transplanting of seedlings in hydroponic) and continued for more than six weeks (Fig. 4). Measures were taken for plant growth traits such as plant height, leaf number, biomass and chlorophyll content. Genotypes had shown variation in sensitivity to salinity stress along the intensity gradient and time of exposure to stress. Combined differential scoring of these parameters enabled classification of the 20 soybean genotypes into five classes: highly susceptible, susceptible, moderately susceptible, tolerant and highly tolerant to

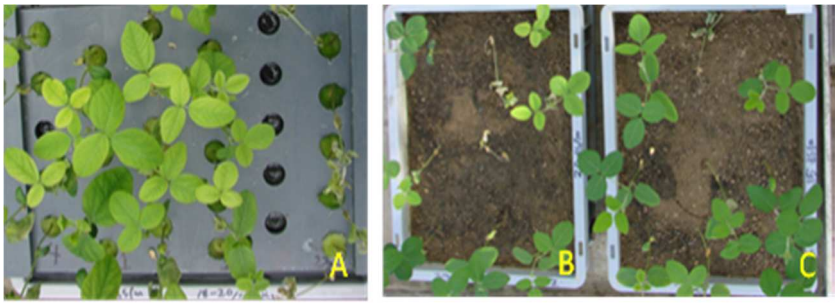

FIG. 4. Screening for salt tolerance in hydroponic with 15 dS/m (A) and confirmatory evaluation in soil (20 and15 dS/m) for same time after salt treatment application (two weeks) (B and C, respectively), each row represent one genotype.

salinity (Table 1). Highly tolerant and tolerant genotypes were confirmed in soil conditions (Fig. 4) and will be further validated under field conditions in Bangladesh. Our tests have shown that extended treatment with different levels of salinity in hydroponic solutions enables the classification of the genotypes based on their tolerance threshold level and can be applied to the screening of mutant populations for salt tolerance.

Table 1. Classification of the 20 soybean genotypes according to salt sensitivity into highly tolerant (I), tolerant (II), moderately susceptible (III), susceptible (IV) and highly susceptible (V)

	Number of genotypes in each salt sensitivity class				
Salt concentration	I	II	III	IV	V
15 dS/m for 2 Weeks	9	7	2	2	-
15 dS/m for 4 Weeks	2	3	6	7	2
15 dS/m for 6 Weeks	2	-	-	1	17
10 dS/m for 4 Weeks	8	7	4	1	-
10 dS/m for 6 Weeks	2	2	7	4	5
5 dS/m for 6 Weeks	7	11	2	-	-

Screening for drought tolerance in cowpea

Mutation breeding has a great potential to induce genetic variability and generate drought tolerant varieties. The PBGL is actively involved in developing efficient and reliable screening methods to induce and identify drought tolerant varieties. Screening packages are being designed and evaluated to suit different crops under different conditions in the laboratory, glasshouse and open field using either a hydroponic system with an osmotic stress generator, usually polyethylene glycol (PEG6000), or a water stress system in soil with controlled irrigation.

FIG. 5: Screening for drought stress using PEG6000 in hydroponic (left) and soil (right). Cowpea plants in hydroponic for two weeks before stress treatment (A), after one week in PEG6000 (B), measurement of chlorophyll fluorescence using flourPen (C) and stomatal conductance using Porometer (D)

During 2015 an experiment was conducted for drought screening in cowpea from Senegal. Two popular cowpea varieties along with four advanced mutant lines (M5) from each variety were used for drought screening using both systems (hydroponic vs soil). PEG6000 was used at 0, 10 and 15% concentrations in the hydroponic system with Hoagland's nutrient solution. Drought stress was imposed at two weeks after germination and continued for a week followed by stress removal to assess the potential for recovery from drought stress among genotypes (Fig. 5). The same genotypes were screened for drought tolerance in pot experiments in the glasshouse. Plants were sown in pots containing 3.5 kg of soil and

irrigated every two days with 200ml of water/pot. Two weeks after germination, drought treatment was applied in the form of complete water stoppage for 20 and 30 days followed by re-watering every two days for one week to assess recovery potential among genotypes. Each treatment was replicated three times in a completely randomized design. Measurements were taken for plant growth, biomass, stomatal conductance and chlorophyll fluorescence until the harvest of the materials. The cowpea varieties and the mutant lines showed variation in sensitivity to drought stress under both hydroponic and soil conditions and in the potential of recovery under the soil system. This variation enabled classification of the genotypes into 'drought susceptible', 'moderate' and 'tolerant'. The next step will be field validation of the tested material in Senegal in order to assess the correlation to field screening under natural drought stress.

Mutation discovery

Nucleotide variation is the major contributor to heritable phenotypic variation. Methods to uncover nucleotide variation provide important information on plant evolution and enable methods for efficient breeding that avoid gene–environment (GxE) interactions. Tools for whole genome sequencing have been rapidly improving to the point where resequencing of hundreds to thousands of plant genomes is now a reality. A drawback of new technologies, however, is that they tend to be expensive and require a high level of technical expertise. New tools, therefore, are not available to all laboratories. Yet, powerful methods can be developed that are low cost and suitable for laboratories with varying infrastructure. One example is the starting point of all genotyping experiments: the extraction of DNA from plant tissues. While long-term storage of plant tissues prior to DNA extraction often involves the use of liquid nitrogen and -80°C freezers, these can be avoided by desiccating and storing leaf material in silica gel at room temperature. Extraction of high quality genomic DNA from leaf material is typically performed using expensive kits, or with more manual methods that require toxic organic chemicals such as the cetyltrimethylammonium bromide (CTAB) method. These can be avoided by binding genomic DNA to silica in the presence of chaotropic salts. This mirrors the chemistry used in expensive kits, but at about 1/10th of the price. Importantly, high quality genomic DNA can be made without specialized equipment for tissue grinding and without the use of any toxic organic compounds that require specialized waste disposal. Finally, nucleases used in assays to discover mutations can be self-extracted at a cost of less than 0.06 cents per reaction.

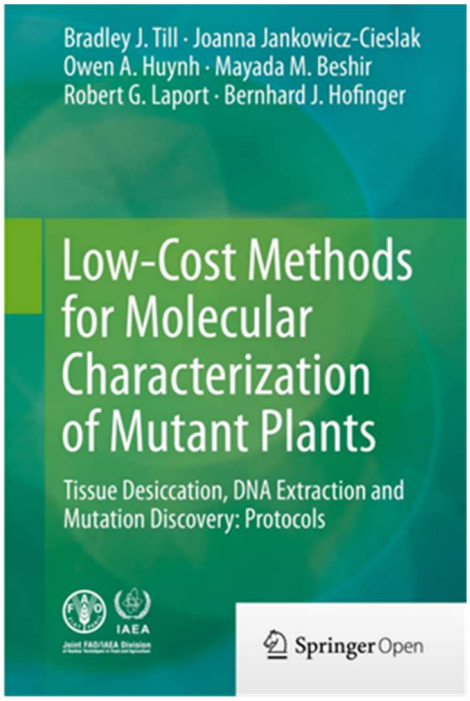

FIG. 6: The open access book from the PBGL provides detailed protocols for low-cost and non-toxic desiccation of plant tissues, extraction of genomic DNA, extraction of single-strand-specific nucleases and enzymatic mismatch cleavage for mutation discovery

In 2015 the PBGL published a book containing easy-to-follow protocols for all of these procedures. The book, titled "Low-Cost Methods for Molecular Characterization of Mutant Plants" was published by Springer and is open access (Fig. 6). It is free to download at http://rd.springer.com/book/10.1007%2F978-3-319-16259-1. The PBGL has already trained more than 100 scientists from over 30 Member States on the methods published in the open access book.

While the publication of the protocol book was in itself a major accomplishment, it does not mean that methods cannot be further improved. PBGL continued the efforts for protocol development throughout 2015. For example, in April 2015 the University of Hyderabad, India, held its 1st National

Workshop on TILLING in Crop Plants. The course organizer, Professor Ramesh Sharma, invited the PBGL to teach the recently published low-cost methods. During the course, at the suggestion of university staff, several modifications were tested. First, the efficiency of using tungsten carbide balls versus standard metal balls was evaluated in tissue grinding. While expensive, tungsten carbide balls can be washed and reused for years. However, they are not available in all countries. Steel balls such as those used in ball bearings of bicycles can be found almost anywhere and purchased in bulk for less than 0.05 cents per ball. At this price, balls need not necessarily be reused, saving time and money also on washing. Data shows that similar results are achieved with each type of ball (Fig. 7). The use of sodium iodide as a chaotropic salt to replace potassium iodide was also tested and shown to have equivalent performance (Fig. 7). Sodium iodide is approximately half the cost of potassium iodide. The chaotropic salt is the most expensive component of DNA extraction and thus switching to sodium iodide marks a major cost reduction. These methods have now been tested on over 20 plant species.

The next step in the evaluation of DNA of mutant plants is to try to find mutations and then to determine if these mutations are associated with improved traits. In 2015 the PBGL devoted its research and development efforts to foster and improve methods for the discovery of different types of mutations. Single nucleotide polymorphisms (SNPs) are the most common type of nucleotide variation found in nature and the predominant type of variation induced by some mutagens. The PBGL has previously developed methods for the extraction of single-strand-specific nucleases that can be used in low-cost mutation discovery assays. In 2015 PBGL optimized this protocol so that enzyme can be prepared in a single day without toxic chemicals using a variety of different plant materials. This protocol reduces assay costs to less than one cent per sample. The draft protocol was prepared and used in training courses in 2015, with an open access publication of the protocol planned for 2016.

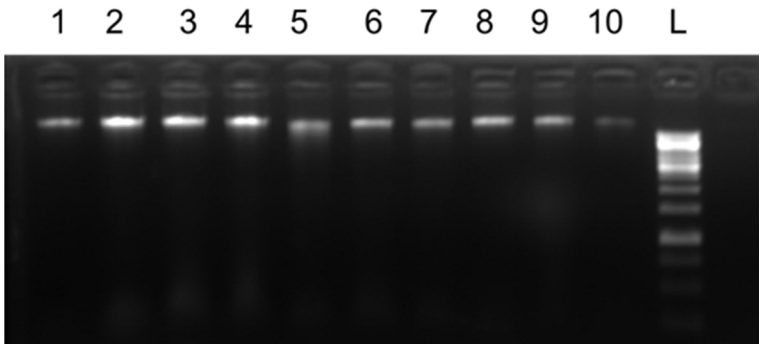

FIG. 7. Example of genomic DNA quality produced at the 1st National Workshop on TILLING in Crop Plants held at the University of Hyderabad in April 2015. Odd lanes represent tissue ground with tungsten carbide balls and extracted using potassium iodide. Even lanes were prepared with steel balls and sodium iodide. Fresh tissue was used for lanes 5 and 10. Tissue desiccated with silica gel was used for all other samples.

Much effort has gone towards the discovery of SNP mutations. However, it can be argued that the discovery of larger genomic aberrations is much more challenging. This is because plants accumulate larger mutations at a much lower frequency than smaller ones. The fewer mutations there are, the harder they are to discover. Further, special techniques are often required to discover such mutations and the optimal technique can change depending on the type of mutation that is induced. Improvements to DNA sequencing technologies are promising to change that. In collaboration with partners at the University of California, Davis, the PBGL is developing methods for whole genome analysis for the recovery of mutations induced by treatment with gamma irradiation. We began this work in September as part of an ad hoc group training course where 17 mutant rice lines were sequenced (see Capacity Building section below). Preliminary analysis suggests that mutations induced by gamma irradiation can be effectively recovered from rice (see Fig. 8). Based on these results the PBGL is developing a guideline for marker development. Putative indels need to be validated and more experiments are planned in 2016 to test this approach in different crops. The next steps will be to develop a genetic crossing strategy that can be used to validate the markers for use in breeding (introgression and marker-assisted selection).

Knowledge of the nucleotide variation that exists in a population prior to mutagenesis is also important. New methods can be developed to rapidly evaluate nucleotide diversity in plants. In 2015 the PBGL began collaboration with the International Center for Tropical Agriculture (CIAT) on the genotyping of cassava accessions held at CIAT. Financial support for this work is provided by the Colombian granting agency, Colciencias. Cassava (*Manihot esculenta*) is an important staple crop for over 500 million people in the tropics. Working with Professor Hernan Ceballos the goal is to characterize cassava for nucleotide variation in genes important for starch quality and herbicide tolerance. DNA was shipped from Colombia to Seibersdorf where it was quantified and pooled prior to screening using an amplicon based next generation sequencing approach. Sequencing will be performed in early 2016. In addition to learning more about genetic variation in cassava, the PBGL is developing streamlined methods for processing thousands of samples. Pilot experiments show rapid quantification of cassava genomic DNA using paramagnetic beads (see Fig. 9).

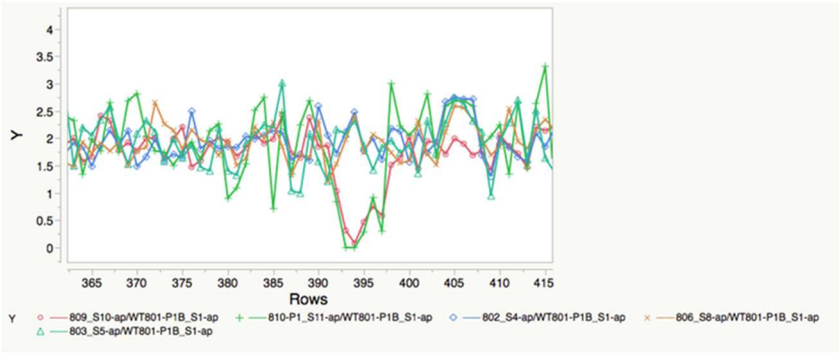

FIG. 8. Graphical data of whole genome sequencing of rice mutants created by seed irradiation with gamma rays. Binning analysis across one chromosome is shown. Two siblings show a decrease in DNA reads suggestive of a homozygous deletion of approximately 150,000 base pairs.

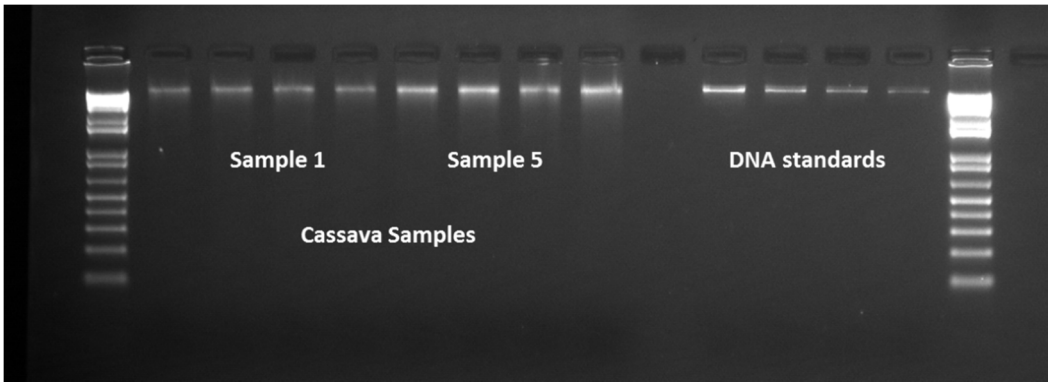

FIG. 9. Agarose gel image showing the concentration of cassava genomic DNA can be rapidly normalized with the use of limited capacity binding paramagnetic beads

Together, the work done in 2015 aimed to build both low cost and higher throughput tools to help Member States improve their mutation breeding programs. Work was carried out based on the demand of the researchers coming to Seibersdorf for training. In terms of molecular biology work, the two most common requests are for methods for characterization of natural germplasm and for methods to efficiently discover mutations that are causing the improved traits observed in the field. Progress made in 2015 suggests that improved methods can be provided for many crop species.

CAPACITY BUILDING

Plant Mutation Breeding and Efficiency Enhancing Techniques

A two-week regional training course on *Phenotyping and Genotyping of Mutants for Abiotic Stresses in Cereals* took place at the PBGL from 5-16 October 2015. Eleven participants (Iraq (3), Jordan (3), Saudi Arabia (2), Syrian Arab Republic (1), Oman (1), and Palestine (1) in the regional Technical

Cooperation (TC) project RAS/5/058 on *Supporting Mutation Breeding Approaches to Develop New Crop Varieties Adaptable to Climate Change* attended the training course as did three scientific visitors from national TC projects in Côte d'Ivoire, Kenya and Laos, as well as six PBGL fellowship trainees from Albania, Bangladesh, Eritrea, Madagascar, Sudan and Syrian Arab Republic. The course included lectures and practical sessions on mutation breeding procedures, tissue culture techniques, genetics and physiology of abiotic stress resistance/tolerance, methodologies for screening of mutant populations, identification and detection of mutants for abiotic stress tolerance, utilization of appropriate technologies for mutant phenotyping and genotyping, low cost genomic DNA extraction and mutation discovery.

Training course participants

On demand training

PBGL staff provided group training in Seibersdorf and served as experts at training courses organized by external institutions, as follows:

20-24 April 2015, Hyderabad, India. Low-cost methods were taught at 1st National Workshop on TILLING in Crop Plants.

11-12 May 2015, Seibersdorf. Ad hoc training course on bench-top enzyme purification and mutation discovery. Participants from Austria and Poland attended this course.

24-28 August 2015, Seibersdorf. Ad hoc training course on whole genome sequencing of rice. Participants from Colombia, Jordan, India and Poland attended the course.

28-30 September 2015, Seibersdorf. Ad hoc training course on chemical mutagenesis of barley seed. Participants from Germany, Syria and Colombia attended the course.

11-12 November 2015, Seibersdorf. Ad hoc training course on low-cost methods for bench-top purification of enzymes and mutation discovery. Participants from Albania, Madagascar, Thailand, Pakistan, Syria and Colombia attended this course.

16-20 November 2015, Nanning, China. Low-cost methods were taught at a training course organized at the Guangxi Academy of Agricultural Sciences.

14-18 December 2015, Hyderabad, India. Low-cost methods and next generation sequencing were taught at the 2nd National Workshop on TILLING in Crop Plants

Fellowships, Scientific Visitors and Interns

Many fellowship candidates desire training at the PBGL since the whole spectrum of mutation breeding, from mutation induction, screening techniques for various characters including abiotic stresses, modern tissue culture techniques to basic and advanced molecular mutation discovery techniques, are practiced here. In 2015, the PBGL hosted 15 fellows from 11 Member States for periods of two to seven months, ten scientific visitors from ten Member States, six interns from five Members States and one cost-free expert from China. Details are given below:

Name	Country	Status	Duration	Topic
Saraye, Banumaty	Mauritius	Fellow	3 months	Heat stress screening of tomato mutants

Name	Country	Status	Duration	Topic
Gueye, Ndiogou	Senegal	Fellow	4 months	Mutation induction in sesame and cowpea, drought screening *in vivo* and *in vitro*
Deme, Ndeye Fatou	Senegal	Fellow	5 months	Mutation screening in cowpea
Kabbia, Milton	Sierra Leone	Fellow	7 months	Cassava mutation breeding
Promnart, Udompan	Thailand	Fellow	4 months	Mutation breeding in rice, screening for abiotic stress tolerance
Rabefiraisana, Harimialimalala Jhonny	Madagascar	Fellow	3 months	Mutation detection in maize and rice
Kaewcheenchai, Reunreudee	Thailand	Fellow	3 months	Mutation detection in rice
Gledjan, Caka	Albania	Fellow	2 months	Heterozygosity tests in tomato
Nzoumbou-Boko, Romaric	Central African Republic	Fellow	4 months	Mutation detection in cassava
Gado Yamba Kassa, Geralde	Central African Republic	Fellow	4 months	
Malek, Mohammad Abdul	Bangladesh	Fellow	4 months	Mutation induction in soybean and screening for salt tolerance
Tecleghiorghis, Kidane	Eritrea	Fellow	3 months	Mutation induction in barley and screening for salt tolerance
Omer, Kamal	Sudan	Fellow	4 months	Mutation induction in wheat and sorghum. Doubled haploid methods
Elias, Rana	Syrian Arab Republic	Fellow	3 months	Mutation induction and detection in barley
Jawdat, Dana	Syrian Arab Republic	Fellow	2 months	Mutation detection in barley and cotton
Taassob Shirazi, Farzaneh	Islamic Republic of Iran	SV	4 months	Mutation induction in barley, screening and accelerated breeding

Name	Country	Status	Duration	Topic
Jouhar, Mohammed	Syrian Arab Republic	SV	2 week	Development of low cost method for mutant characterization
Szurman-Zubrzycka, Miriam	Poland	SV	1 week	Low-cost purification of single-strand-specific nucleases for mutation discovery
Bannister, Stephanie	Austria	SV	1 week	
Kihara, Amin	Japan	SV	1 week	Screening methods for drought and salt tolerance
Rahman, Mehboob-Ur	Pakistan	SV	2 weeks	Mutation detection technologies
Fadila, Ziani Eps Abed	Algeria	SV	2 weeks	ARASIA regional training course on 'Phenotyping and genotyping of mutants for abiotic stresses in cereals'
Boualaphanh, Chanthakhone	Lao P.D.R.	SV	2 weeks	
Kouadio, Justing Yatty	Côte d'Ivoire	SV	2 weeks	
Nyongesa, Albert	Kenya	SV	2 weeks	
Chen, Zhiwei	China	Cost Free Expert	7 months	Mutation induction and identification in rice
Sochacka, Anna	Poland	Intern	6 months	Plant mutation induction, *in vitro* propagation and screening techniques
Datta, Sneha	India	Intern	3 months	Plant mutation induction, *in vitro* propagation and mutation discovery
Kafuri, Lina	Colombia	Intern	4 months	Discovery of natural mutations in cassava
Tello, Daniel	Colombia	Intern	4 months	
Barakat, Abdel	Egypt	Intern	3 months	Mutation detection
Khraisat, Sana'a	Jordan	Intern	3 months	Mutation detection using retrotransposon based markers

SERVICES

The PBGL provides an irradiation service to Member States for mutation induction. In 2015 a total of 30 requests for plant irradiation services from 20 Member States were handled. These are listed below and included 27 crop species. The total number of irradiation requests now stands at 1440. For each request, PBGL carries out radiosensitivity tests to determine the optimal irradiation dose for mutation induction. We therefore normally request that Member States send us sufficient seed for this initial test (usually 100–300 seeds). Once the optimal dose has been determined, this is applied to the rest of the seed samples and the M1 seeds returned to the Member State.

The PBGL has developed positive control kits to assist Member States in optimizing PBGL protocols in their own laboratories and for their particular plant species. Each kit contains a detailed protocol along with the materials needed to complete this protocol. Kits are available upon request. The three kits were distributed to the following Member States in 2015:

- Low cost DNA extraction kit: India, Austria, Jordan, Mauritius, Pakistan;
- Low cost enzyme extraction kit for mutation discovery: Austria, India, Iraq, Jordan, Mauritius, Oman, Pakistan, Poland, South Africa, Syria;
- Low cost mutation discovery kit (now replaced by the above low cost enzyme extraction kit): India.

Irradiation services provided to Member States in 2015

Request no.	Country	Crop
1411	USA	Pea, bean
1412	UK	*Rudbeckia fulgida* (orange coneflower)
1413	Germany	Ornamental
1414	Madagascar	Cassava
1415	Botswana	Maize
1416	Spain	Euphorbia
1417	UK	Wheat
1418	Laos	Rice, soybean, mung bean, maize
1419	Nepal	Rice
1420	Mongolia	Wheat, rapeseed
1421	Namibia	Rice
1422	Palestine	Durum wheat
1423	Nigeria	Sesame
1424	Nigeria	Coffee

Request no.	Country	Crop
1425	Sri Lanka	Mung bean, soybean, onion, hot pepper
1426	Côte d'Ivoire	Maize
1427	Spain	Rice
1428	UK	Wheat
1429	UK	Rapeseed
1430	Nigeria	*Digitaria exilis* (fonio)
1431	Kenya	*Dolichos lablab*, *Brachiaria ruziziensis*
1432	Nigeria	*Vigna vexillata*
1433	Sierra Leone	Cassava, cocoa, peanut
1434	Oman	Wheat, barley
1435	Iraq	Hot pepper
1436	Oman	Wheat, barley
1437	Austria	Wheat
1438	Côte d'Ivoire	Barley
1439	Morocco	Lentil
1440	Kenya	*Dolichos lablab*, *Brachiaria ruziziensis*

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EXTERNAL COLLABORATIONS AND PARTNERSHIPS

Institution	Topic
International Center for Tropical Agriculture (CIAT), Cali, COLOMBIA	Detection of mutation events in South American 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); protocol development for salt tolerance testing
International Network for the Improvement of Banana and Plantains (INIBAP), Bioversity International, Montpellier, FRANCE	Induced mutations in *Musa* for tolerance to biotic stresses
Austrian Institute of Technology, Health & Environment Department, Tulln, AUSTRIA	Gene expression profiling in drought
University of Natural Resources and Life Sciences, Tulln, AUSTRIA	Methods on marker assisted breeding; Near-infrared spectroscopy (NIRS) analysis in characterising mutant seed phenotypes; Mutants for barley fodder, quality testing

Institution	Topic
University of Agriculture, Department of Plant Physiology, Krakow, POLAND	Banana phenotyping for drought tolerance
University of Natural Resources and Life Sciences, Department of Biotechnology, Vienna, AUSTRIA	Induced and natural mutation induction in crop plants, including under-studied crops; Statistical data evaluation
Agri-Science Queensland, Hermitage Research Facility, Warwick, AUSTRALIA	Barley crossing method; barley mutant stocks and mutation breeding
The James Hutton Institute, Invergowrie, Dundee, Scotland, UK	Barley crossing method; barley genetic stocks, genetic markers for low lignin mutants
University of Dundee, Scotland, UK	Molecular genetics of lignin mutants for fodder barley
Nordic Genetic Resource Center, Alnarp, SWEDEN	Classic barley mutants, mutant gene descriptions and nomenclature
UC Davis Genome Center, Davis, California, USA	Developing next generation sequencing strategies for discovery of induced mutation events in genomes of seed and vegetatively propagated crops
University of Ljubljana, SLOVENIA	X-ray irradiation for mutation induction; pollen irradiation for haploid production
Arid Land Research Center, Tottori University, Japan	X-ray irradiation for mutation induction; Phenotyping for abiotic stress tolerance
Agricultural Research Corporation, Khartoum, Sudan	Phenotyping of mutant populations for development of protocols for X-ray irradiation for mutation induction
Ruđer Bošković Institute, Zagreb, CROATIA	Ion beam irradiation
Department of Molecular Systems Biology, University of Vienna, AUSTRIA	Metabolomics of mutant crops
John Innes Centre, Norwich, UK	Reverse genetics in grass pea
Bench-Bio, Vapi City, Gujarat, INDIA	Reverse genetics in grass pea
Mature Citrus Biotechnology Facility, Citrus Research and Education Center, University of Florida, Lake Alfred, USA	Citrus reverse genetics

Institution	Topic
Gregor Mendel Institute of Molecular Plant Biology, Vienna, AUSTRIA	Adaptation to climate change
University of Sydney, AUSTRALIA	Disease resistance in wheat
Agriculture and Agri-Food Canada, Winnipeg, Manitoba, CANADA	Disease resistance in wheat
The Genome Analysis Centre, Norwich, UK	Disease resistance in wheat
The Sainsbury Laboratory, Norwich, UK	Disease resistance in wheat
Department of Plant Pathology, Stellenbosch University, Matieland, SOUTH AFRICA	Disease resistance in banana
Du Roi Agritech (Pty) Ltd, Letsitele, Greater Tzaneen Rural, SOUTH AFRICA	Disease resistance in banana
Taiwan Banana Research Institute, Pingtung, Taiwan, China	Disease resistance in banana

THE SOIL AND WATER MANAGEMENT & CROP NUTRITION LABORATORY

EXECUTIVE SUMMARY

The Soil and Water Management & Crop Nutrition Laboratory (SWMCNL) is part of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. It assists Member States in the development and transfer of isotopic and nuclear technologies to improve the resilience of farmers' communities to climate change and variability by protecting soil and water resources and optimizing soil, water and nutrient management practices. The SWMCNL also helps Member States to be better prepared in responding to nuclear emergencies affecting food and agriculture, as well in remediating the impact of these events on soil and agricultural water resources.

In 2015, the SWMCNL carried out a wide range of activities: (i) Develop and validate robust and affordable isotope, nuclear and related conventional techniques for climate-smart agriculture; (ii) Support the improvement of nuclear emergency response in food and agriculture, (iii) Train technical staff and scientists from Member States in the use of nuclear and related techniques to develop improved and integrated soil-nutrient-water-plant management practices; (iv) Conduct isotope analyses to projects where analytical facilities are not available; and (v) Provide quality assurance services to Member States.

The research and development activities at the SWMCNL in 2015 included the improvements in the use of caesium-137 for assessing long-term erosion, in particular in fragile upland environments, nitrogen-15 for reducing greenhouse gas emission, the combined use of carbon-13 and nitrogen-15 for assessing soil organic carbon sequestration, and cosmic-ray soil moisture neutron probe for area-wide soil moisture assessment. In addition, new activities were initiated in the field of soil conservation through the use of compound-specific stable isotope analysis to identify sediment pathways and areas prone to land degradation. All activities have made important progress and are essential in supporting the implementation of the Coordinated Research Projects of the Soil and Water Management and Crop Nutrition Subprogramme.

A second major component of the work of the SWMCNL is its contribution to capacity building in Member States. The SWMCNL hosted 30 fellows from 19 countries, each receiving a two-week, intensive training on the application of isotopic and nuclear techniques to improve nitrogen or agricultural water management in support of climate-smart agriculture. The SWMCNL also supported a two-week regional training course on the use of fallout radionuclides for assessing soil redistribution in agroecosystems, in Morocco attended by 21 fellows from nine African Member States.

An IAEA publication, *Supporting Sampling and Sample Preparation Tools for Isotope and Nuclear Analysis* (IAEA-TECDOC-1783) was published. The publication contains five Standard Operating Procedures providing illustrated, step by step guidance for scientists, technicians and students on sampling procedures and tools for isotope and nuclear analysis for soil and water management.

Information was further communicated to Member States through 21 publications as book chapters, conference papers and publications in international peer-reviewed journals. With 2015 being the International Year of Soils, several documents were published, including the *Vienna Soil Declaration: Soil Matters for Humans and Ecosystems*.

In 2015, the SWMCNL analysed a total of 4585 and 180 samples for stable isotopes and fallout radionuclides, respectively. Most analyses were carried out in support of R&D activities in the SWMCNL focusing on the design of isotope and nuclear techniques to improve soil and water management practices. Twenty percent of all stable isotope analyses were performed for the Plant Breeding and Genetics Laboratory, and the Insect Pest Control Laboratory, which are both laboratories of the Joint FAO/IAEA Division.

STAFF

Name	Title
Dercon, Gerd	Laboratory Head
Mabit, Lionel	Soil Scientist
Wahbi, Ammar	Soil Scientist
Aigner, Martina[1]	Senior Laboratory Technician (50%)
Heiling, Maria	Senior Laboratory Technician
Weltin, Georg	Senior Laboratory Technician
Resch, Christian	Senior Laboratory Technician
Toloza, Arsenio	Laboratory Technician
Gruber, Roman[2]	Laboratory Technician
Jagoditsch, Norbert	Technical Attendant
Mayr, Leo[3]	Consultant
Albinet, Franck[4]	Consultant (Extra-budgetary)
Adzigogov, Lazar[4]	Consultant (Extra-budgetary)
Mletzko, Joanna Malgorzata	Team Assistant
Krukle, Agneta[5]	Intern
Slaets, Johanna[6]	Intern
Yan, Tiezhu[6]	Intern
Torres Astorga, Romina[7]	ICTP Fellow
Grabenhofer, Jutta[8]	Visiting Scientist (Cost-free)

[1] Retired in February 2015 after nearly 30 years of service at the SWMCNL; [2] Joined the SWMCNL in October 2015; [3] Stayed for two months at the SWMCNL; [4] Joined the SWMCNL as home-based consultant from December 2014 to May 2015; [5] Stayed at the SWMCNL from December 2014 to March 2015; [6] Joined the SWMCNL in October 2015; [7] Stayed at the SWMCNL from September to December; [8] Joined the SWMCNL in November 2015.

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

The Soil and Water Management and Crop Nutrition Laboratory (SWMCNL) assists Member States in the development and transfer of isotopic and nuclear technologies to improve the resilience of farming communities to climate change and variability by optimizing soil, water and nutrient management practices. These efforts are supported by a new generation of robust and affordable isotope and nuclear techniques that can be used *in situ* at the plot (on-farm) or at the area-wide level.

The SWMCNL also supports Member States to be better prepared in responding in nuclear emergencies affecting food and agriculture, as well as in remediating the impact of such events on soil and agricultural water resources.

Climate-Smart Agriculture

Climate change is a major threat to food security. Changes in weather patterns, with increasing severity of storms, floods, droughts and extreme temperatures, impact sustainable agricultural production. These result in soil erosion, land degradation, increased greenhouse gas emission and crop failures worldwide. The need to sustain agricultural production in these challenging conditions has never been greater. Consequently, there is an increasing demand from Member States for technical assistance and training in developing soil and water management packages for climate change mitigation and adaptation.

Use of compound-specific stable isotope techniques to determine sediment origin in a small Austrian agricultural catchment

Land degradation currently affects 1.9 billion hectares globally or about 65 per cent of global soil resources. Of this, 85% is due to soil erosion. As a result, as much as 75 billion tonnes of fertile soil is lost from world agricultural systems each year through this process. The economic cost associated with on-farm and off-farm soil erosion is estimated at US $400 billion per year. The ability to measure the magnitude and sources of soil erosion will help to control this through efficient conservation practices. In 2015, the SWMCNL tested the compound-specific stable isotope (CSSI) techniques in a three-hectares agricultural catchment at Mistelbach, situated 60 km north of Vienna (Fig. 1), to determine the source of eroded soil and thereby identify areas prone to soil degradation. The CSSI techniques enables the determination of sediment origin and contribution from different land uses by linking fingerprints of land use to sediment in the deposition zones. The fingerprints used are organic biomarkers, such as natural fatty acids, and their stable isotope signature of carbon-13 (^{13}C). Communities of plant label the soil where they grow by exuding these fatty acids, whose stable isotopic signature is different for each plant species.

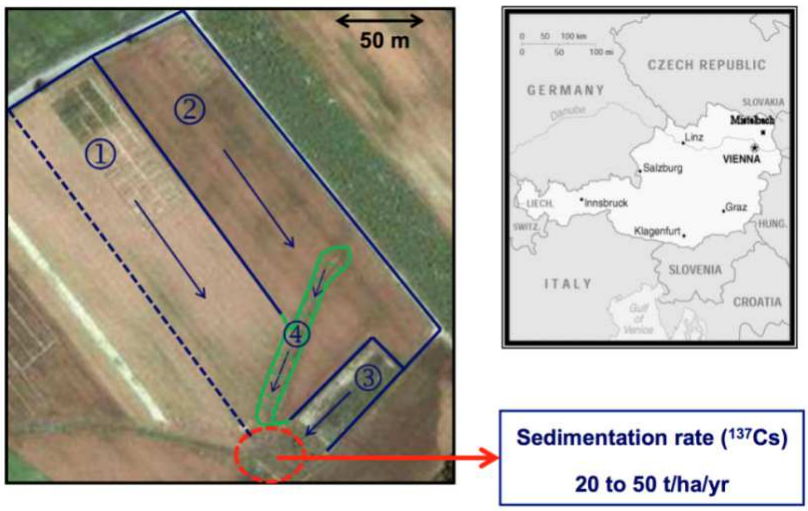


FIG. 1: Location of catchment at Mistelbach, Austria, showing sampling positions (four agricultural fields [sources: 1-4]) and sedimentation area (red)

Using fallout radionuclide (FRN) techniques (i.e. caesium-137), the SWMCNL had preciously established

sedimentation rates of 20-50 t ha^{-1} yr^{-1} in the lowest part of this same catchment, showing high erosion far above soil tolerance levels. The CSSI techniques (based on soil samples from four main contributing agricultural fields within the catchment) allowed quantification of the different soil sources (Fig. 1). The study identified one major source (source 4 - the main waterway of the catchment) contributing 55% to the sediment deposited at the catchment outlet, whereas sources 1, 2 and 3 contributed 4, 15 and 26%, respectively.

This study highlights that CSSI and FRN techniques are complementary tools as fingerprints and tracers of sediments in the landscape. While FRN techniques provided information on the sedimentation magnitude at the outlet of the studied catchment, CSSI techniques applied for fatty acids identified the origin of those sediments. Jointly applied, these isotopic techniques can provide key information to land managers to facilitate sustainable land management, particularly important as climate change is expected to further accelerate soil erosion.

This research has been conducted within a new Coordinated Research Project (CRP) D1.50.17 on *"Nuclear Techniques for Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems".*

Combined use of the caesium-137 technique and the Revised Universal Soil Loss Equation to assess snow-gliding impact on soil erosion in steep Alpine environments

Under CRP D1.50.17 on *"Nuclear Techniques for Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems"*, the SWMCNL collaborated with the Environmental Geosciences team of Basel University (Switzerland) on the combined use of caesium-137 and conventional monitoring and modelling techniques to understand soil erosion processes in steep Alpine environments. Erosion studies carried out previously have mostly neglected the role and contribution of snow cover on soil erosion rates. This raises the question whether annual snow cover and particularly the slow movement of snow packages over the soil surface (i.e. snow gliding) contribute significantly to total soil loss in these areas.

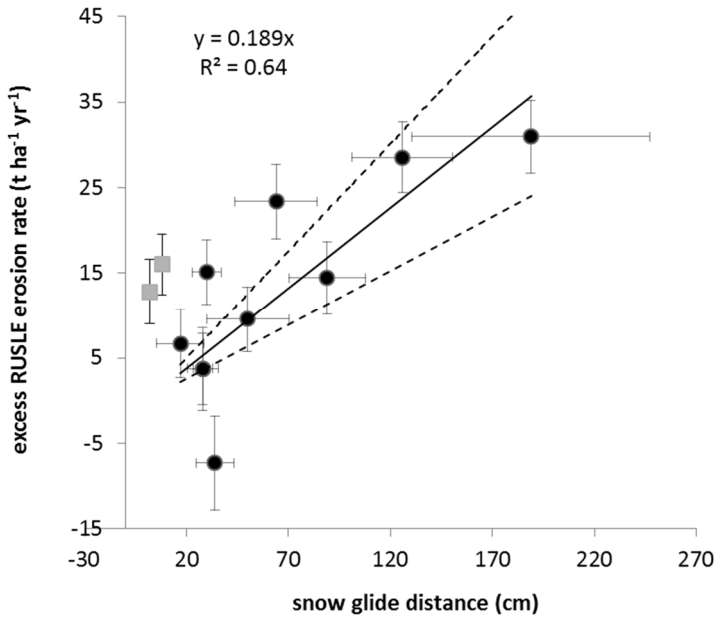


FIG. 2: Correlation of the cumulative measured snow glide distances (cm) versus the difference of the ^{137}Cs and RUSLE soil erosion rates (t ha^{-1} yr^{-1}) for the grassland sites (dots, n=10) and shrub sites A1N, A2N (squares, n=2). Y-error bars represent the error of both the ^{137}Cs and RUSLE estimates. X-error bars represent the standard deviation of replicate snow glide measurements at one site. Solid line represents a linear regression and the dotted lines the 95% confidence interval.

Three different approaches were tested to estimate soil erosion rates at 14 experimental sites with two different land uses (grassland and large shrub [*Alnus viridis*]), located in the Ursern Valley of central Switzerland: (i) the ^{137}Cs technique that integrates soil loss due to all erosion agents involved, (ii) the Revised Universal Soil Loss Equation (RUSLE) that is suitable for the estimation of soil loss by water erosion and (iii) direct sediment yield measurements of snow glide deposits. Moreover, cumulative snow glide distance was measured for the 14 sites.

The mean ^{137}Cs based soil erosion rates of 17.8 t ha^{-1} yr^{-1} are approximately four times higher than the average RUSLE estimates. Congruent with RUSLE, the ^{137}Cs-

based average soil erosion rate on the north facing slopes is lower than on the south facing slopes (by 8.7 t ha^{-1} yr^{-1}). The observed difference between the ^{137}Cs and RUSLE based soil erosion rates, which can be interpreted as snow glide-triggered soil erosion, is correlated to the measured snow glide distance (Fig. 2). With increasing snow glide rates an increase of soil loss is observed. The estimated magnitude of the excess RUSLE erosion rate corresponds well with the magnitude estimated from the sediment yield measurements.

The application of RUSLE and ^{137}Cs proved the relevance of the snow glide process for a longer time scale. Additionally, it highlighted that for an accurate soil erosion prediction in high mountain areas it is crucial to assess and quantify the erosivity of snow movements. Both approaches indicate that snow gliding is a major soil erosion agent in steep snow-covered mountain grasslands. Surface roughness may reduce snow glide rates, particularly on the in general more intensely used south facing slopes. This is an important result with respect to soil conservation since surface roughness can be modified through an effective land use management in these specific environments.

A simple statistical approach to evaluate the uncertainty around the mean level of caesium-137 fallout at undisturbed reference sites

One of the major issues related to the use of ^{137}Cs as a soil erosion/sedimentation tracer is the selection of an undisturbed reference site to determine the initial ^{137}Cs fallout input or reference inventory. The initial ^{137}Cs fallout input is a key component of the conversion models used to estimate erosion and sedimentation rates from the ^{137}Cs data set. Understanding the uncertainty of the initial ^{137}Cs fallout amount at these reference sites is hence essential for determining the accuracy of the derived erosion estimations.

As the number of cores collected at a reference site is often limited for logistic reasons, the SWMCNL developed a simple statistical approach for evaluating the uncertainty around the mean level of ^{137}Cs fallout at undisturbed reference sites based on the number of samples taken. Derived from the equation to calculate the minimum number of soil cores required to provide a reliable mean estimate of the reference inventory within a specified level of confidence, equation (1) was able to assess the allowable error (AE) at a selected confidence level for the number of samples already collected as follows:

$$AE = \frac{t_{(\alpha,\ n-1)} \cdot CV}{\sqrt{n}} \qquad (1)$$

where:

AE: allowable error (decimal fraction);
t: t value of the Student's t-test at a selected percentage of confidence (e.g. for 90% α = 0.1);
n: number of soil samples collected;
CV: coefficient of variation (decimal fraction).

The usefulness of this equation was demonstrated at the experimental research station of the Austrian Agency for Health and Food Safety, Grabenegg, 100 km west of Vienna. In a flat undisturbed permanent pasture (i.e. a stable reference site), nine soil cores were collected to evaluate the initial ^{137}Cs fallout, and measured to be 7890 ± 1510 Bq m^{-2} (mean ± SD) with a coefficient of variation (CV) of 19.2%. With nine samples taken and a CV of 19.2%, the evaluated ^{137}Cs mean baseline inventory of 7890 Bq m^{-2} was established with an allowable error (AE) of 12% at a 90% confidence level.

This simple statistical test can be applied in other fallout radionuclide (FRN) based assessment (e.g. beryllium-7, excess lead-210 or plutonium-239+240) and can provide clear information about the accuracy of the mean value of FRN estimated at any selected reference site.

This research was conducted under the CRP D1.50.17 on *"Nuclear Techniques for Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems"* and D1.50.15 on *"Response to Nuclear Emergency Affecting Food and Agriculture"*.

Laser carbon-13 and nitrogen-15 isotope analysis for greenhouse gases implemented at SWMCNL

Agriculture, forestry and other land use (AFOLU), contribute 20-24% to the total anthropogenic emission of greenhouse gases (GHG) such as nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4). This contribution makes the AFOLU globally the largest emitting sector after energy, and even more important in developing countries[1]. IPCC estimates annual GHG emissions (mainly CH_4 and N_2O) from agricultural production in 2000–2010 at 10–12% (5.0–5.8 Gt CO_2 eq/yr), and annual GHG flux from land use and land use change activities at 9–11% of global emissions (4.3–5.5 Gt CO_2 eq/yr). The same IPCC report indicates that a combination of supply and demand options can reduce by up to 80% the emissions from the sector by 2030.

To develop technologies for greenhouse gas emission reduction in agriculture, it is imperative to have the expertise and capability for measuring and tracing GHG to get further insights about their sources in soil. For this purpose, in 2015, the SWMCNL acquired two laser isotope analysers to measure carbon-13 and the nitrogen-15 signatures of CO_2 and N_2O (Fig. 3). These analysers are now being tested and calibrated under field and laboratory conditions.

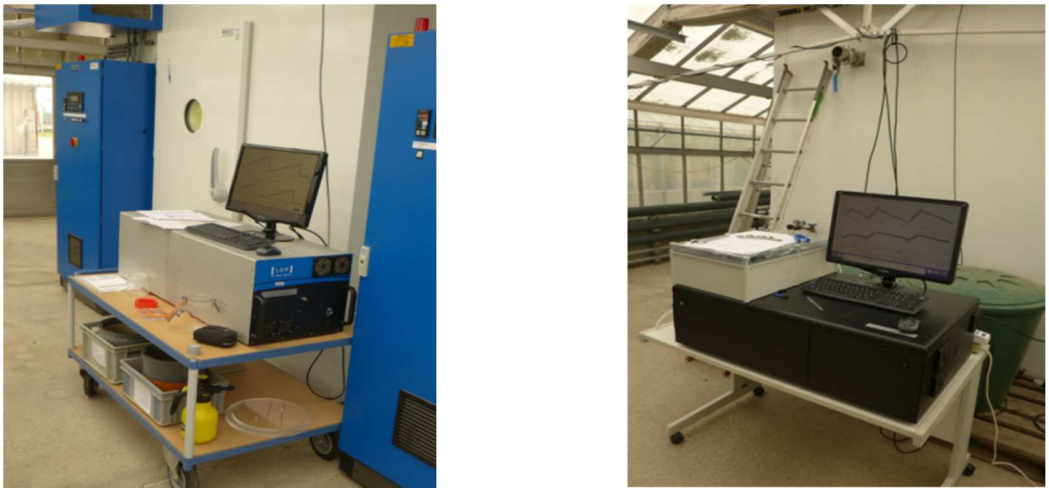


FIG. 3: (left) Nitrogen-15 N_2O laser isotope analyser for screening the efficiency of N process inhibitors in reducing N_2O emission from urea-fertilizer applied to lysimeters in growth chamber (left); (right) Controlling carbon-13 enrichment of CO_2 in the growth chamber during carbon-13 labelling of maize (right).

The acquisition of this equipment is also linked to CRP D1.50.12 on *"Soil Quality and Nutrient Management for Sustainable Food Production in Mulch-based Cropping Systems in Sub- Saharan Africa"* and CRP D1.50.16 on *"Minimizing farming impacts on climate change by enhancing carbon and nitrogen capture and storage in Agro-Ecosystems"*. The SWMCNL aims to play a key role in providing technical support to Member States in using stable isotopic technique of ^{15}N and ^{13}C at natural abundance to unveil the C-N interaction to optimise both C and N capture as well as to reduce GHG.

FIG. 4: Carbon-13 labelling of maize in ^{13}C-CO_2 analyser controlled walk-in growth chamber at the SWMCNL

Preliminary tests have been made on the use of the

[1] IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

^{13}C-CO_2 analyser (Fig. 4) in carbon dynamics studies. It is also being used in the optimization of our walk-in growth chamber for ^{13}C labelling of plant materials (maize) (Fig 4). This analyser helps to stabilize the ^{13}C enrichment of the CO_2 in the growth chamber to ensure homogeneity of plant labelling. The ^{13}C-labelled material is then used in organic carbon decomposition studies to trace the source of CO_2.

Analysis of the labelled plant material (about 1 kg of dry matter) showed a maximum ^{13}C enrichment of 370‰, as targeted in the design, with homogeneous labelling in top and middle leaves (Table 1). Enrichment was lower, however, in the older leaves. This systematic variation, together with measured ^{13}C-CO_2 dynamics over time in the growth chamber, will be addressed to further improve^{13}C-labelling of plant material.

Table 1: Measured carbon-13 enrichment (‰) in three different positions of maize plants (confidence interval 95%)

Position	Average	Lower Limit	Upper Limit
Top leaves (n=28)	366.3	362.7	369.9
Middle leaves (n=28)	341.9	331.8	351.9
Bottom leaves (n=28)	277.2	246.8	307.6

Protocol development for continuous nitrogen-15 measurement of N_2O and its isotopomers for real-time greenhouse gas tracing

Quantifying sources of nitrous oxide is essential to improve our understanding of the global N cycle and to develop climate-smart agriculture, as N_2O has a global warming potential 300 times higher than CO_2. The isotopic signature and the intramolecular distribution (site preference) of ^{15}N are powerful tools to trace N_2O, but the application of these methods is limited as conventional methods cannot provide continuous and *in situ* data.

We have developed a protocol for continuous (closed-loop), real time monitoring of the N_2O flux, the isotopic signature and the intramolecular distribution of ^{15}N by using off-axis integrated cavity output spectroscopy (ICOS, Los Gatos Research). The method was applied in a fertilizer inhibitor experiment, in which N_2O emissions were measured on undisturbed soil cores for three weeks. The treatments consisted of enriched urea-N (100 kg urea-N/ha), the same fertilizer combined with the nitrification inhibitor nitrapyrin (375 g/100 kg urea), and control cores.

Monitoring the isotopic signature makes it possible to distinguish emissions from soil and fertilizer. Characterization of site preference could additionally provide a tool to identify different microbial processes leading to N_2O emissions. Furthermore, the closed-loop approach enables direct measurement on-site and does not require removal of CO_2 and H_2O.

Results showed that 75% of total N_2O emissions (total=11 346 µg N_2O-N/m^2) in the fertilized cores originated from fertilizer, while only 55% of total emissions (total=2 450 µg N_2O-N/m^2) stemmed from fertilizer for the cores treated with nitrapyrin (Fig. 5). In the controls, N_2O derived from soil was only 40% of the size of the corresponding pool from the fertilized cores, pointing towards a priming effect on the microbial community from the fertilizer and demonstrating the bias that could be introduced by relying on non-treated cores to estimate soil emission rates, rather than using the isotopic signature.

The site preference of ^{15}N in N_2O (defined as the numeric difference between $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$**)** increased linearly over time for the cores with fertilizer and those with nitrapyrin. However, the increase was stronger for the fertilized cores: during the first 10 days, these cores showed a more

negative site preference than the cores with inhibitor, while during the last 10 days, the site preference for the fertilized cores was more positive than that of the inhibitor. This change indicates that the site preference of ^{15}N can be used to distinguish the processes of nitrification and denitrification, the former having been supressed by nitrapyrin in the cores treated with the inhibitor. Low enrichment levels (5% atomic excess in this study) sufficed in order to separate emissions from soil and fertilizer, making the proposed closed-loop approach a cost-effective and practical tool to obtain a continuous, *in situ* characterization of N_2O sources.

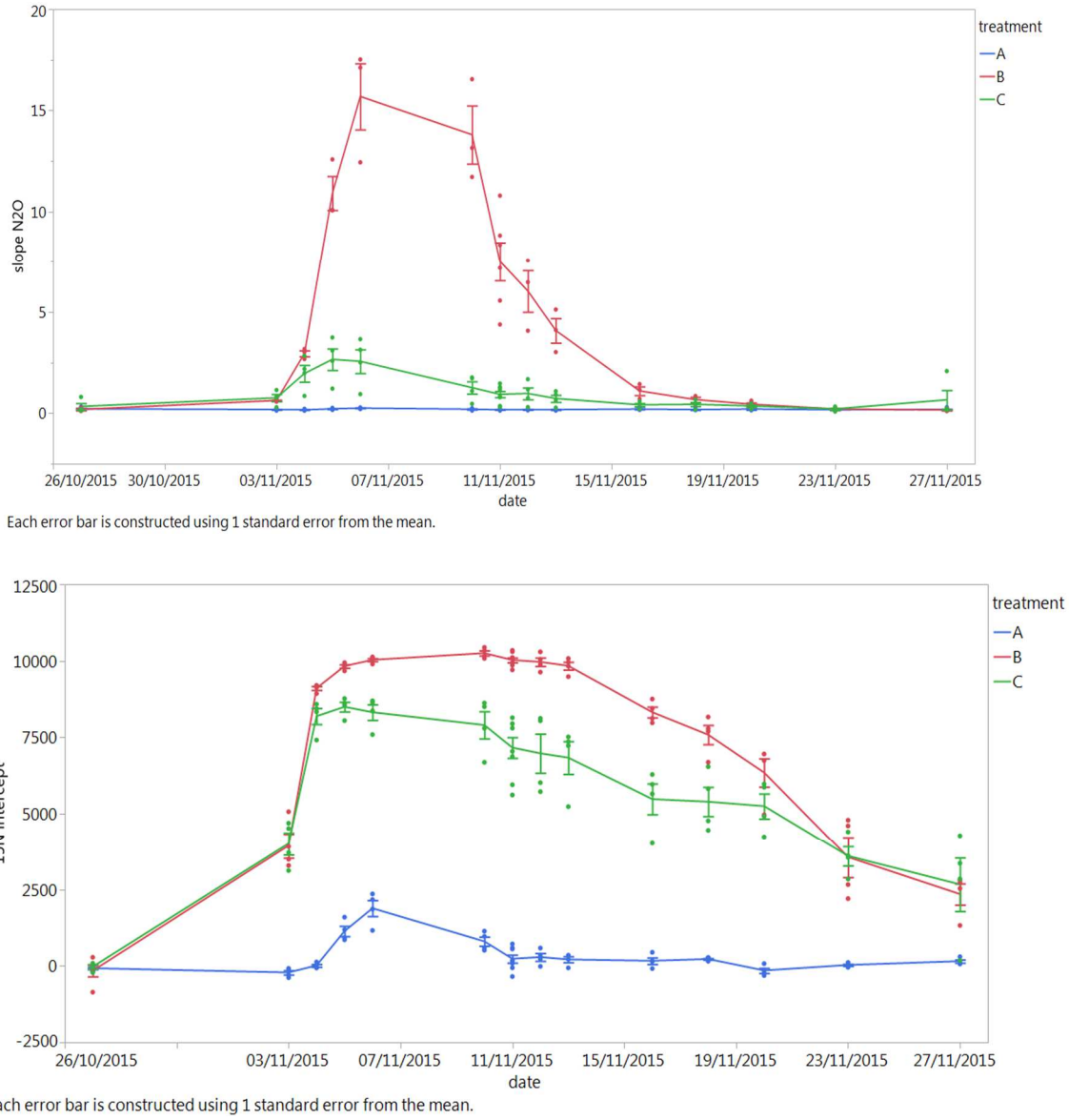


FIG.5: (top) Total N_2O emissions (ppm) and (bottom) ^{15}N-N_2O signature (‰) over time for undisturbed cores without (treatment A) and with urea fertilizer (without inhibitor (treatment B) and with inhibitor (treatment C))

This research was conducted within the CRP D1.50.16 on *"Minimizing farming impacts on climate change by enhancing carbon and nitrogen capture and storage in agro-ecosystems"*.

Combining old and new stable isotope techniques to evaluate the impact of conservation tillage on soil organic carbon dynamics and stability

Soil organic matter (SOM) is a major carbon pool. It is a crucial factor for soil quality, including several soil physical properties, and a major nutrient source for crops. It also plays a significant role

in the global carbon cycle. Soils can act as a carbon sink or source depending on land use and agricultural management practices. Some practices such as conservation tillage or no-tillage could increase SOM stocks, particularly in the topsoil, but in the long term it remains to be seen if and how this SOM is stabilized. In order to evaluate the efficiency and sustainability of soil carbon sequestration measures and the impact of different management and environmental factors, information on SOM stability and mean residence time (MRT) is required. This information may be expensively determined via radiocarbon dating, precluding a widespread use of stability measurements in soil science; alternative methods based on stable carbon and nitrogen isotopes can provide this information at a fraction of the cost.

As part of the CRP on *"Soil Quality and Nutrient Management for Sustainable Food Production in Mulch-based Cropping Systems in Sub-Saharan Africa"* (D1.50.12), we used two stable isotope methods developed by Balesdent and Balabane (1992) [2] and by Conen *et al.* (2008) [3] in efforts to develop a cheap and accessible technique to determine the stability of SOM. As a first step, both techniques were used to assess the impact of long-term conservation tillage on SOM distribution, dynamics and stability on four long-term conservation tillage experiments in Belgium (Fig. 6).

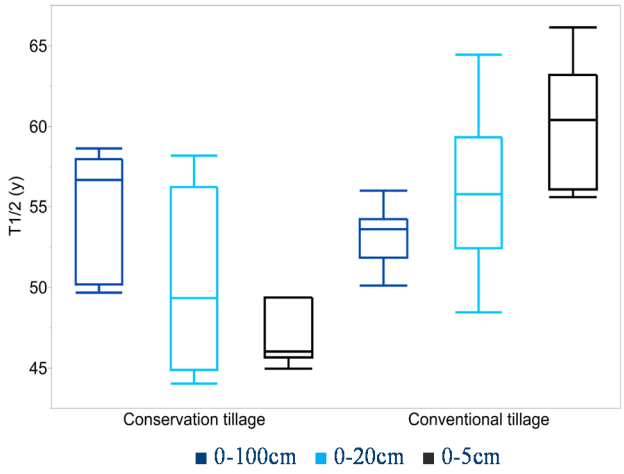


FIG 6: Half-life of SOM (in years) calculated according to the Balesdent and Balabane method for one of the four sites.

For each till and no-till treatment of each selected experiment, six replicates of 1m soil cores were sampled and divided into 8 different depth layers. Samples from depths of 0-5cm, 10-15cm, 40-60cm were divided into SOM and aggregate classes. The samples from all depth layers, bulk soil and fractions, were analysed with an elemental analyser coupled to an IRMS for carbon and nitrogen content and their stable isotope ratios.

The soil column sampling showed a significant increase in organic carbon content (%) in the 0-5cm soil layer of the conservation tillage treatment and a slight increase in the total carbon content down to 1m. This increase was mainly concentrated in the particulate organic matter and the protected micro-aggregate fractions. The relative stability of the SOM was calculated in three soil layers using the ^{15}N fractionation method. A clear increase in SOM relative stability could be seen with increasing depth and no significant difference was found between till and no-till, however, a trend towards decreasing stability with conservation tillage was observed.

When using the method developed by Balesdent and Balabane on one of the sites that had experienced a C_3 to C_4 crop shift, a decreased half-life of the SOM in the top 5cm of the conservation tillage treatment was found (Fig. 6). This decrease corresponds with the trend observed using the Conen method. Individually the methods were not powerful enough, but combining all measured parameters in a multivariate principle components analysis allowed discriminating between sampling depth, crop input and land use (till vs no till) systems and getting an indication of the SOM stability.

[2] Balesdent, J., Balabane, M. (1992). Maize root-derived soil organic carbon estimated by natural 13C abundance. Soil Biol. Biochem. 24:97-101.

[3] Conen, F., Zimmermann, M., Leifeld, J., Seth, B., Alewell, C. (2008). Relative stability of soil carbon revealed by shifts in δ15N and C:N ratio. Biogeosciences 5:123–128.

The next step will be to develop a usable model based on the changes in $\delta^{13}C$, $\delta^{15}N$ and C and N concentrations in different soil fractions, essentially combining the two stable isotope approaches, to accurately and cost effectively determine SOM stability.

For more information, see de Clercq *et al.* (2015). Predicting soil organic matter stability in agricultural fields through carbon and nitrogen stable isotopes. Soil Biol. Biochem. 88, 29–38.

Can we screen phosphorus movement in the landscape through the analysis of $\delta^{18}O$ isotopic abundance in phosphate?

The SWMCNL explored the possibility of using $\delta^{18}O$ isotopic signature in phosphate for screening phosphorus (P) movement in the landscape. Phosphorus is essential for crop production, but extensive use of P fertilizer and animal manure can lead to eutrophication of rivers and lakes. In order to study these effects, numerous studies on P movement in the soil plant system and P transformation processes have been performed in the past decades. Assessing losses of P through erosion processes, however, is almost impossible – particularly at the landscape level and on a longer timescale. Using the isotopic signature of stable oxygen isotope ^{18}O in the phosphate ion as a tracer could be a cost-effective and widely applicable way to study P movements. This approach is already applied as a paleo-temperature proxy (the fractionation between phosphate and water is temperature dependent) and can be used for quantifying P losses through leaching into surface and groundwater, as oxygen exchange between phosphate and water is slow in the absence of biological activity.

The aim of this study was to test if using the isotopic signature of stable oxygen isotope ^{18}O in the phosphate ion as a tracer would be feasible to screen P movements from uplands to lowlands or if the effect of microorganisms would prohibit the application. Several biochemical reactions lead to a shift of $\delta^{18}O$ in phosphate. Supposedly microorganisms preferentially take up lighter isotopologues of phosphate, leading to an enrichment of heavier isotopologues in the residual phosphate. Several enzymatic processes, which are necessary for living organisms to avoid phosphate toxication, lead to oxygen exchange from the surrounding water.

The silver phosphate method was applied in Petzenkirchen in the foothills of the Alps in Lower Austria and in Rauris, located in the national park Hohe Tauern in the Alps. Manure and soil samples of different altitudes were collected and processed for silver phosphate analyses.

The $\delta^{18}O$ in phosphate values had very similar signals in Petzenkirchen (14.69-15.09‰ $\delta^{18}O$ in soil and 13.77-15.23‰ $\delta^{18}O$ in manure samples), with no significant difference between different

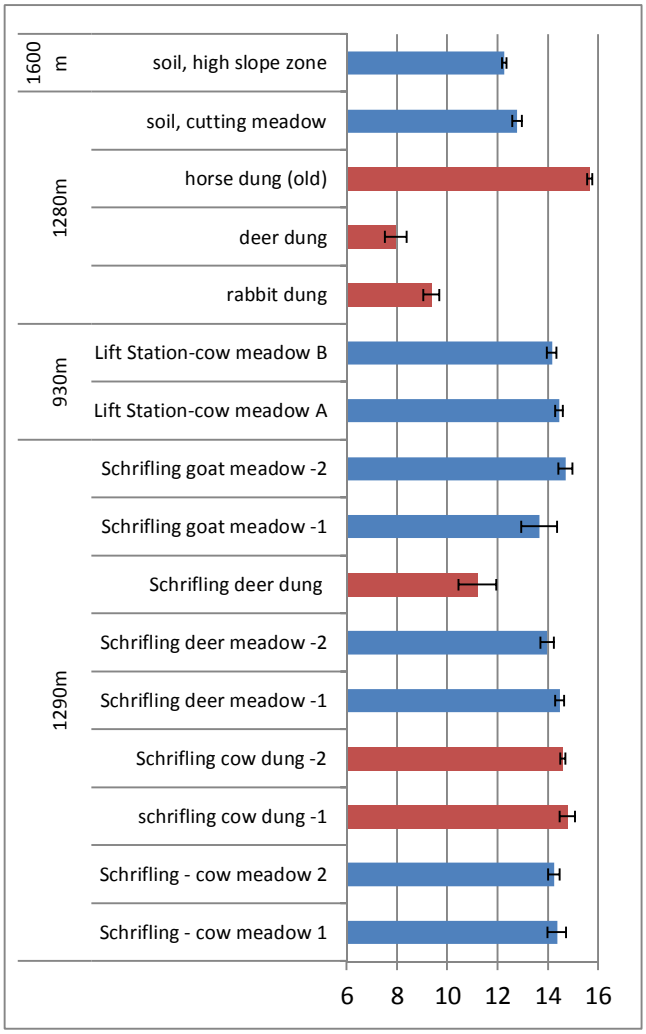


FIG. 7: $\delta^{18}O$ (‰) in phosphate values in manure and soil samples of different altitudes at Rauris, Austria

locations within Petzenkirchen. While the number of replicates was too small to show significant differences between the different soils at the Rauris site, at this location a depletion of ^{18}O of 2‰ $\delta^{18}O$ was observed with increasing altitude (Fig. 7). This decrease could be due to different isotopic oxygen composition of snow compared to rain water or because of different microbial communities and/or different microbial activities. Therefore, the silver phosphate method could be an important tool to study biological processes influencing the P cycle. While the $\delta^{18}O$ value of phosphate itself cannot be used to trace phosphate sources within sites, the results indicate additional potential for distinguishing processes that differ with altitude and climatic factors, which could be important when quantifying the effects of climate change on phosphorus cycling.

This research was conducted under CRP D1.20.12 on "*Optimizing Soil, Water and Nutrient Use Efficiency in Integrated Cropping-Livestock Production Systems*".

Protocol development for using cosmic-ray neutron probe to monitor soil water content at landscape level

We also developed a generic protocol on the use, calibration and validation of the cosmic-ray neutron probe (CRNP) to monitor soil water content at landscape level, in close collaboration with Hydroinnova, the University of Nebraska, USA, the Technical University of Vienna and the Federal Agency for Water Management, Austria. The CRNP is a passive and non-invasive technique to monitor water content in the top 50 cm of the soil, covering an area of about 30 hectares. If successful, such large-scale soil moisture content measurements could prompt major improvements in weather forecasting and irrigation practices, and provide a wealth of new data about land uses and the impacts of climate change.

The protocol development was supported by experimental work at a field site located at the Petzenkirchen research station of the Federal Agency for Water Management and the Technical University of Vienna. At this site, located about 100 km west of Vienna, the SWMCNL operates its CRNP since December 2013.

After a first field calibration campaign of the CRNP in December 2013, further calibration was carried out in 2015 using the method of Bogena *et al.* (2013)[4] for determining soil water content (SWC) under different soil water conditions, cropping patterns and stages (Table 2). For this purpose, soil samples were collected for gravimetric water content determination in July, August and October 2015. The area-wide average soil moisture, obtained from the CRNP, was used in this calibration, which was carried out by averaging the large number of soil samples collected within the footprint of 30 hectares of the CRNP.

Based on this gravimetric sampling, the N_0 parameter was also calculated (Table 2). N_0 is a site-specific parameter essential for the calibration of the CRNP. This calibration parameter depends mainly on the physio-chemical characteristics of the surrounding soil. Table 1 shows N_0 values of soil sampling carried out in 2015, compared to the value based on the sampling in 2013. The N_0 values were higher at low gravimetric water content, indicating the importance of determining the N_0 for different SWC or cropping conditions.

Sixteen time-domain transmissivity (TDT) sensors were installed in 2013 at fixed points within the footprint of the CRNP to record hourly volumetric SWC at four depths (0-5 cm, 5-10 cm, 15-20 cm and 45-50 cm). The measurements of these TDT sensors were used to validate CRNP observations of SWC at landscape level. Comparison of the daily data of the landscape TDT with the CRNP measurements showed that root-mean-square error (RMSE) is in the same order of magnitude (~0.02 m^3/m^3) as the RMSE of TDT measurements for the depth of 0-10 cm.

[4] Bogena, H.R., Huisman, J. A., Baatz, R., Franssen, H.J.H., and Vereecken, H. (2013). Accuracy of the cosmic-ray soil water content probe in humid forest ecosystems: The worst case scenario. Water Resources Research, 49, 5778-5791.

Table 2: Calculated N_0 obtained from independent gravimetric sampling in 2013 and 2015 at Petzenkirchen

Date	N readings	Gravimetric water content (g/g)	Calculated N_0	Cropping pattern and stages
11-12 Dec. 2013	2008	0.274	1398	n.a.
2-3 July 2015	2146	0.217	1477	Barley (45%, maturity), maize (45%, stem elongation), and trees (forest; 10%)
27-28 Aug. 2015	2197	0.197	1700	Bare soil (45%), maize (45%, early maturity) and trees (forest; 10%)
28 Oct. 2015	2167	0.236	1659	Rape seed (45%), barley (30%, 2-leaf stage), bare soil (15%), and trees (forest; 10%)

In addition, two time-domain reflectometry (TDR) campaigns were carried out in April 2014 over the CRNP footprint area, which also showed that volumetric SWC was similar to the values measured by CRNP (Fig. 8).

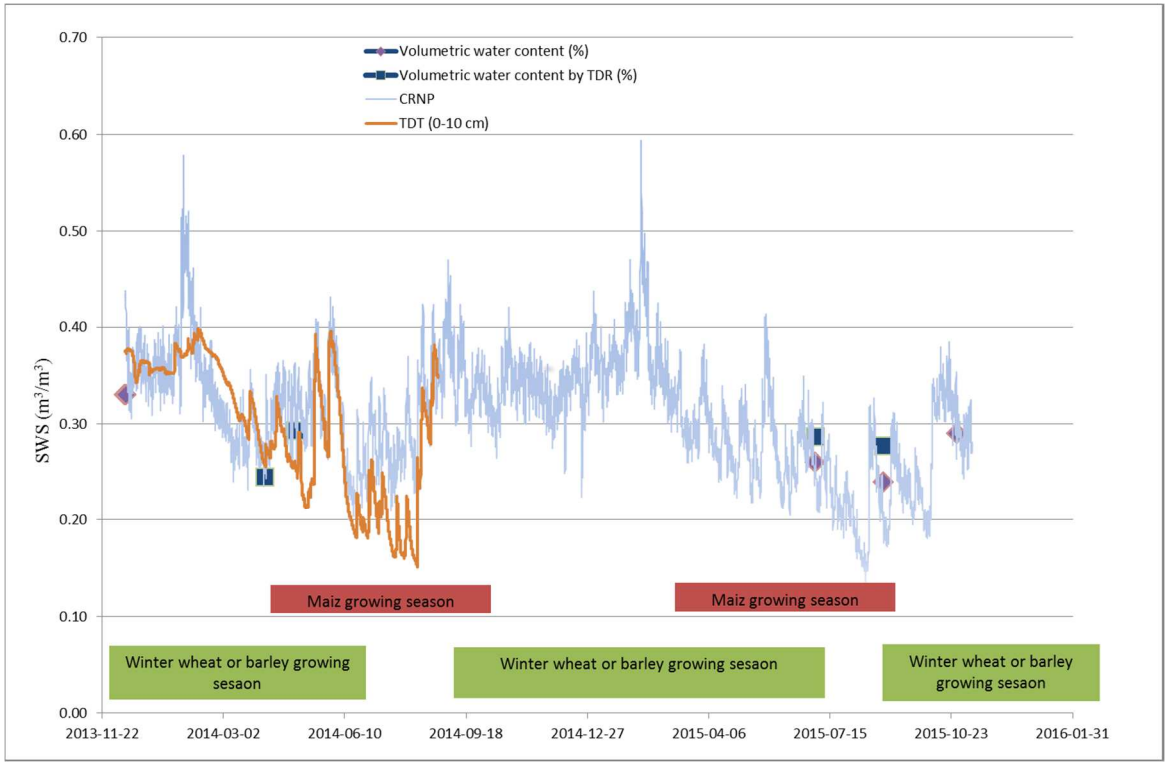


FIG. 8: Time series of site average soil water content (SWC) of time-domain transmissivity (TDT) values by depth, SWC from the cosmic-ray neutron probe (CRNP), and independent gravimetric (12 December 2013, 3 July, 28 August and 28 October 2015) and time-domain reflectometry (TDR) sampling campaigns (5 and 30 April 2014 and 3 July and 28 August 2015) at Petzenkirchen, Austria.

The developed protocol is expected to be published in 2016. This work was carried out under CRP D1.20.13 on *"Landscape Salinity and Water Management for Improving Agricultural Productivity"*.

Measurements by the CRNP can be followed in real-time at http://cosmos.hwr.arizona.edu/Probes/StationDat/087/index.php.

Initiation of a new CRP for developing innovative isotopic techniques and approaches to improve knowledge on the impact of climate change on soil erosion in upland agro-ecosystems

A new CRP, D1.50.17, on "*Nuclear Techniques for a Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems*" was approved at the end of 2015. The CRP aims to (i) develop nuclear techniques to assess impacts of changes on soil erosion and (ii) distinguish and apportion impacts of climate variability and agricultural management on soil erosion in upland agro-ecosystems. Nuclear techniques, including fallout radionuclides (FRNs) such as caesium-137, lead-210, beryllium-7 and plutonium-239 and 240, compound-specific stable isotope (CSSI) techniques based on the measurement of carbon-13 natural abundance signatures of specific organic compounds (i.e. fatty acid) and cosmic-ray soil moisture neutron probe (CRNP) will be used to fulfil the CRP objectives.

Nuclear Emergency Preparedness in Food and Agriculture

Based on recent experience, there is a critical need to improve nuclear emergency preparedness in food and agriculture, including the collection (sampling and analysis), management and visualization of appropriate data from affected areas, for timely dissemination and communication to stakeholders and the general public. Member States are therefore increasingly requesting technical assistance in their endeavours to improve nuclear emergency preparedness and response in food and agriculture.

Response to nuclear emergencies affecting food and agriculture - an online food safety information system for nuclear and radiological emergencies

This CRP D1.50.15 on *"Response to Nuclear Emergency Affecting Food and Agriculture"*, aims to develop and assess systems of innovative data collection (including soil and foodstuff sampling and analysis), management and geo-visualization platforms that can be used for both routine monitoring and emergency response to nuclear and radiological incidents that could affect food and agriculture.

Over the last year, the early prototype of the Online Food Safety Information System was transitioned to a more advanced system linking both data management and visualization components.

The data management component reflects the appropriate workflow: (i) assignments of roles, (ii) data collection tasks management (Fig. 9), (iii) mobile data collection, (iv) data aggregation, and (v) data validation, analysis and sharing.

The data visualization component ensures that data flows smoothly from small to large scale and that appropriate visualization enables analysts and decision makers to gain valuable insights into the data. In addition, a resource analysis tool, "Log Map", was included (Fig. 10), which enables decision-makers to optimize the use of the available resources, such as sample collectors and laboratories. It also provides a direct overview of implementation rate and the efficiency of food sampling campaigns.

Both components have now been integrated, thus providing an advanced prototype receiving growing attention from a variety of partners prior to further testing and validation.

Ultimately, it is envisaged that the system may also be adaptable to other emergency situations involving time-stamped and geo-referenced information, such as plant pest emergencies and transboundary animal diseases.

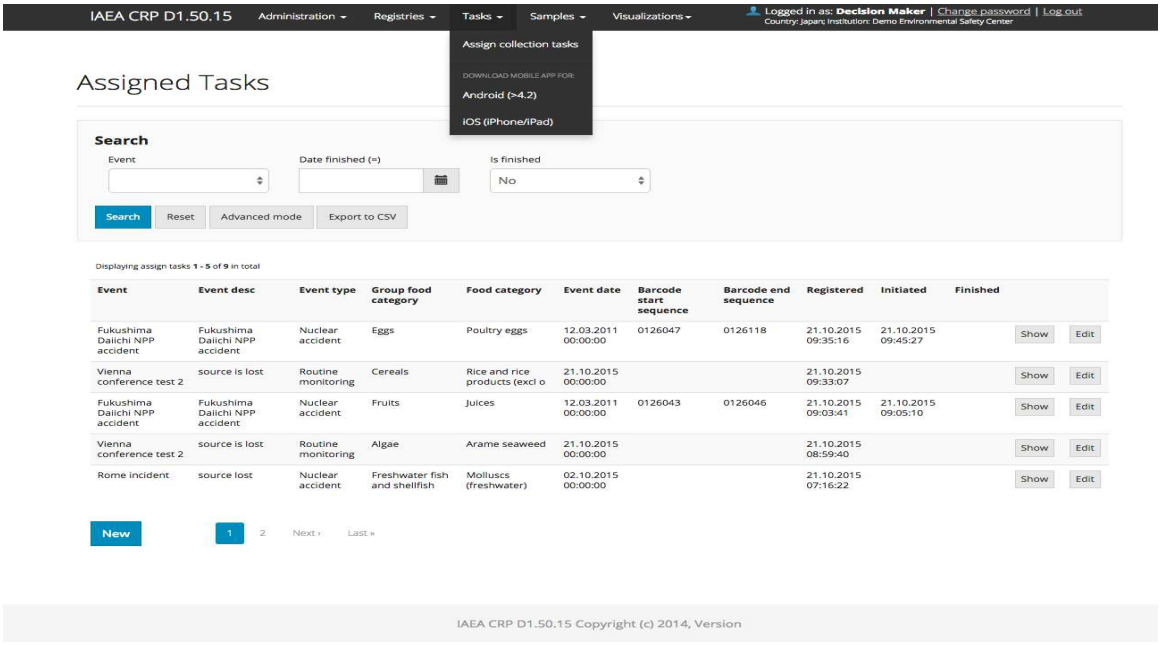


FIG. 9: General overview of platform user interface and task assignment view of the Online Food Safety Information System.

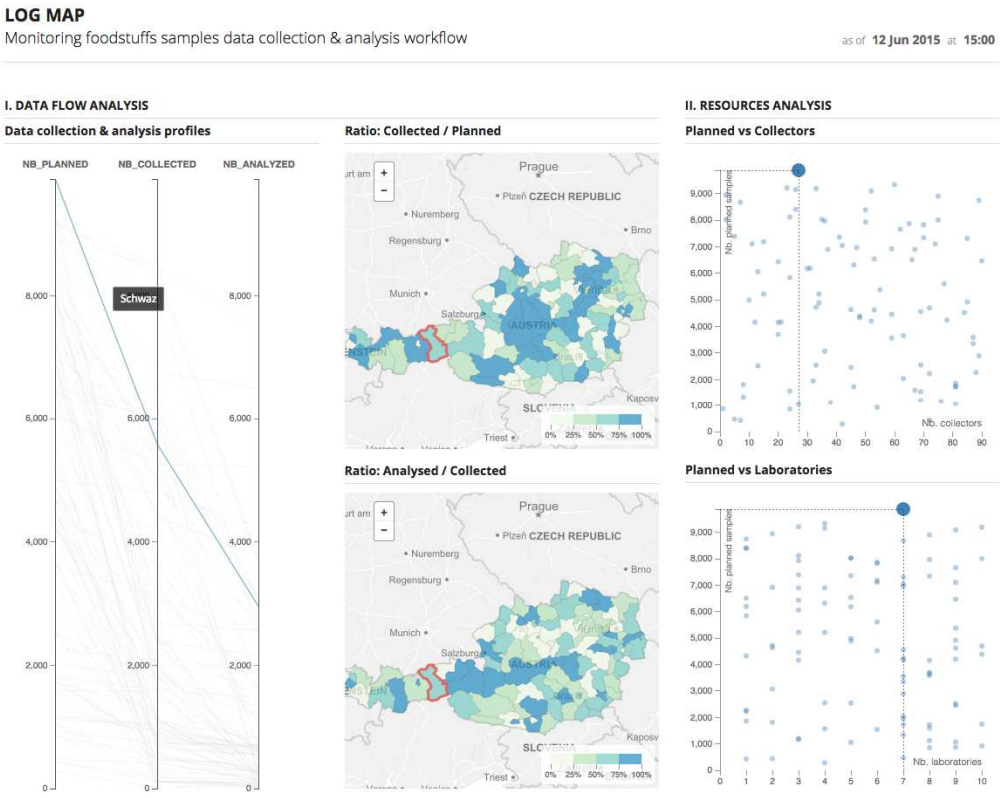
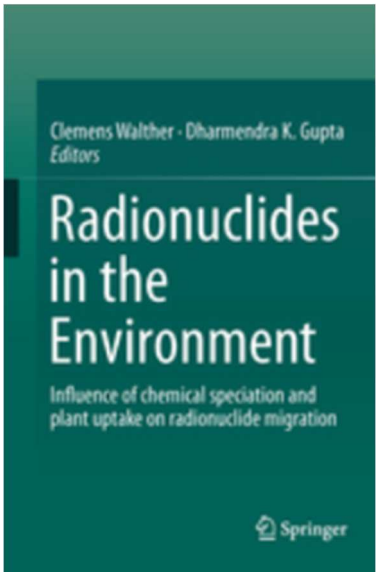


FIG. 10: Example of Log Map monitoring data collection and analysis flows.

Mobility and Bioavailability of Radionuclides in Soils

A review of the processes driving mobility and bioavailability of key artificial and natural radionuclides (e.g. ^{137}Cs, ^{90}Sr, ^{239,240}Pu, ^{241}Am, ^{238}U, ^{226}Ra, ^{232}Th) in different soil types under various environmental conditions was published as a book chapter in "*Radionuclides in the Environment: Influence of chemical speciation and plant uptake on radionuclide migration*" by Springer. The review

highlights that, by changing soil and environmental conditions, radionuclides can be converted from a potentially mobile to an immobile form or vice versa, thereby having a direct effect on their uptake by plants.



The environmental behaviour of radionuclides depends on ecosystem characteristics. A given soil's capacity to immobilize radionuclides has been shown to be the main factor responsible for their resulting activity concentrations in plants. The mobility and bioavailability of radionuclides in soils is complex, depending on clay-sized soil fraction, clay mineralogy, organic matter, cation exchange capacity, pH and quantities of competing cations. Moreover, plant species have different behaviours regarding radionuclide absorption depending on soil and plant characteristics.

In order to develop appropriate strategies that support policy decisions, it is crucial to understand the behaviour of radionuclides in the environment, their potential mobility and bioavailability related to long-term persistence, radiological hazards and impact on human health. Such knowledge is particularly useful in developing long-term remediation and management strategies for terrestrial ecosystems potentially at risk of being contaminated by artificial radionuclides or radionuclides originating from uranium mining legacy sites, with the goal of limiting the transfer of radionuclides to the food chain.

For more information, see Iurian, A.R, Olufemi Phaneuf, M., Mabit, L. (2015). Mobility and Bioavailability of Radionuclides in Soils. In: Radionuclides in the Environment: Influence of chemical speciation and plant uptake on radionuclide migration. Eds: C. Walther and D.K. Gupta. Springer. pp 37–59.

CAPACITY BUILDING

Regional training course on Nitrogen Management Using Isotopic Techniques, 4-15 May 2015, Seibersdorf, Austria

This two-week training course was carried out in the frame of the regional TC project RAS5068 on *Developing Effective Practices for Combating Desertification* and attended by 15 fellows from seven countries (Iraq, Jordan, Lebanon, Oman, Saudi Arabia, Syrian Arab Republic and United Arab Emirates). It focused on the use of nitrogen-15 techniques to assess nitrogen (N) use efficiency as well as emission of nitrous oxide (N_2O) to the atmosphere by agricultural systems, in efforts to develop effective and climate-smart soil-water management and cropping practices. The course also included hands-on collection of soil and plant samples for measuring N use efficiency and gaseous emissions of greenhouse gases under laboratory and field conditions.

Training course on Water Management and Use of AquaCrop Simulation Model, 26 May to 12 June 2015, Seibersdorf, Austria

The main focus of this training course was on: (i) improving water management in rainfed and irrigated agriculture, (ii) monitoring soil water, (iii) calculating soil water balance at field scale, and (vi) how to use the AquaCrop simulation model for improving soil water management and irrigation scheduling. Besides lectures, laboratory and field work, a field excursion was organized to research stations at Grabenegg and Petzenkirchen to demonstrate on-farm research on water management at field and area-wide scales. Fourteen scientists and technicians specialised in irrigation and water

management from six Member States (Bangladesh, Iraq, Italy, Kuwait, Palestine and Sri Lanka) participated in the training.

The training was funded by the IAEA Technical Cooperation Department through several national TC projects.

African regional training course on Fallout Radionuclides Data Treatment and Interpretation with Special Focus on the ^{137}Cs Technique for Assessing Soil Degradation, 28 September – 9 October 2015, Rabat, Morocco

This training course was organised by the Centre National de l'Energie, des Sciences et des Techniques Nucléaires (CNESTEN) of Morocco in close collaboration with the SWMCNL in the frame of regional Technical Cooperation project RAF 5063 on "*Supporting Innovative Conservation Agriculture Practices to Combat Land Degradation and Enhance Soil Productivity for Improved Food Security*").

The objective was to provide participants with advanced knowledge and information on the use of fallout radionuclides (FRN) and how to process and analyse FRN data-sets, with an emphasis on the ^{137}Cs method for assessing soil redistribution in agro-ecosystems. The course covered a wide range of aspects, such as FRN reference site selection, sampling strategy, gamma spectroscopy analysis, dating and FRN conversion models, data modelling and treatment using geographic information systems (GIS), statistics, geostatistics and data treatment and interpretation.

Twenty-one participants from Africa (Algeria, Benin, Ivory Coast, Madagascar, Morocco, Senegal, Tunisia, Uganda and Zimbabwe) attended this training course.

ANALYTICAL SERVICES

The SWMCNL analysed 4585 soil and plant samples for stable isotopes (carbon-13, nitrogen-15, oxygen-18, deuterium) and 180 samples for fallout radionuclides (Caesium-137 and Beryllium-7) in 2015. These samples were mainly from ongoing SWMCNL activities focusing on the design of affordable isotope and nuclear techniques to develop climate-smart agricultural practices (e.g. Soil organic carbon management, soil conservation). Analytical support for isotopic analyses was provided also to the Plant Breeding and Genetics Laboratory and the Insect Pest Control Laboratory of the Joint FAO/IAEA Division, with 654 and 218 samples analysed respectively.

External quality assurance: annual proficiency test on ^{15}N and ^{13}C isotopic abundance in plant materials

The worldwide comparison of stable ^{15}N and ^{13}C isotope analyses provides confidence in the performance of laboratories that measure stable isotopes of N and C in plant tissues. Hence, it is an invaluable tool for external quality control.

The 2015 Proficiency Test on ^{15}N and ^{13}C isotopic abundance in plant materials was again organized by the University of Wageningen, the Netherlands, and funded by the SWMCNL. The Wageningen Evaluating Programs for Analytical Laboratories (WEPAL, http://www.wepal.nl) is accredited by the Dutch Accreditation Council for the organization of inter-laboratory studies.

Every year, one ^{15}N-enriched plant test sample is included in one round of the WEPAL's International Plant-Analytical Exchange program. The ^{15}N-enriched plant sample is sent together with three other plant tissue samples that are not enriched with ^{15}N. Participants are invited to perform analysis of any type offered in the WEPAL International Plant-Analytical Exchange (IPE) program, including ^{15}N (enriched and/or natural abundance), total N (N-elementary), Kjeldahl-N, ^{13}C and total C (C-elementary).

A special evaluation report for IAEA participants on the analytical performance in the stable isotope analysis is issued by the SWMCNL. This evaluation report is sent to the participants together with a certificate of participation.

Participants registered in the program were provided with WEPAL test sample set, IPE 2015.2, which consisted of four samples, each sample containing 20 g of plant material. In total, 13 stable isotope laboratories participated in this round, including laboratories in: Africa (Morocco), Asia (Pakistan and the Philippines [2 laboratories]), Europe (Austria, Belgium and Germany), South America (Argentina, Brazil [2 laboratories], Chile and Uruguay) and the South Pacific (New Zealand).

Nine out of 11 laboratories participating in the N analysis reported ^{15}N-data within the control limits for the enriched plant sample (Fig. 12). Nine out of ten participating laboratories in the C analysis reported ^{13}C isotopic abundance results within the control limits (Fig. 13).

To be prepared for further proficiency tests, 28 kg ryegrass (with a uniformly ^{15}N enrichment of 0.40 atom % excess) and 57 kg barley (with a uniformly ^{15}N enrichment of 0.44 atom % excess) were produced by the SWMCNL. Fourteen kg of each material was sent to the University of Wageningen, for processing.

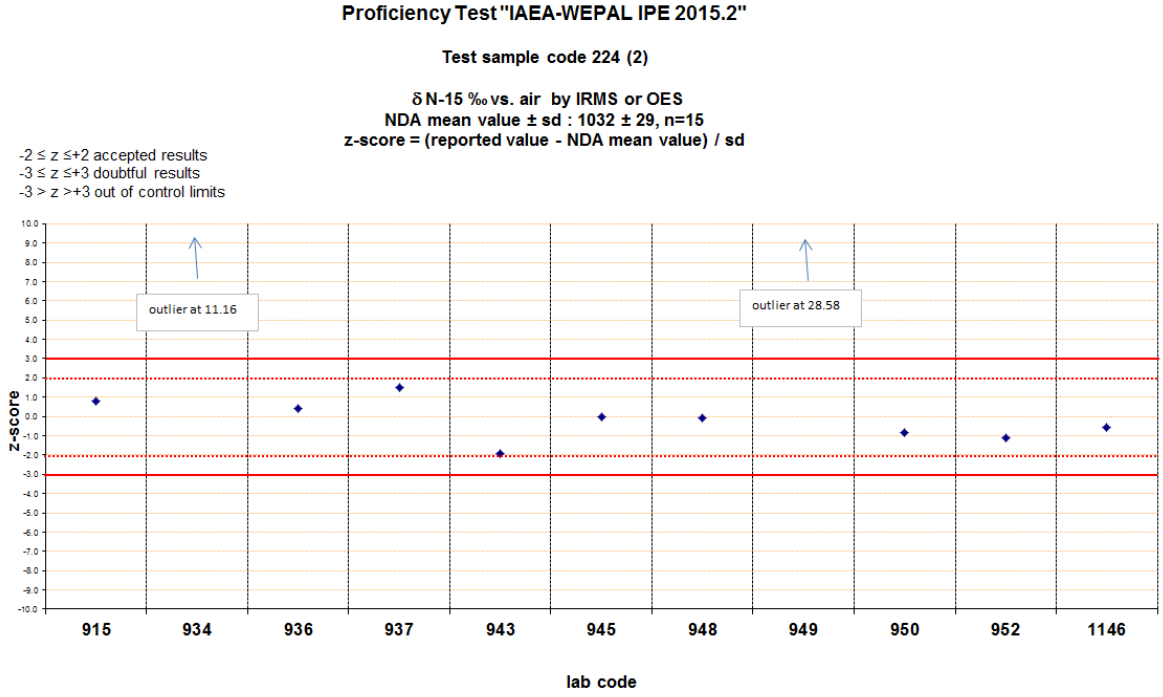


FIG. 12: Z-score evaluation of the ^{15}N analysis in the 2015 proficiency test.

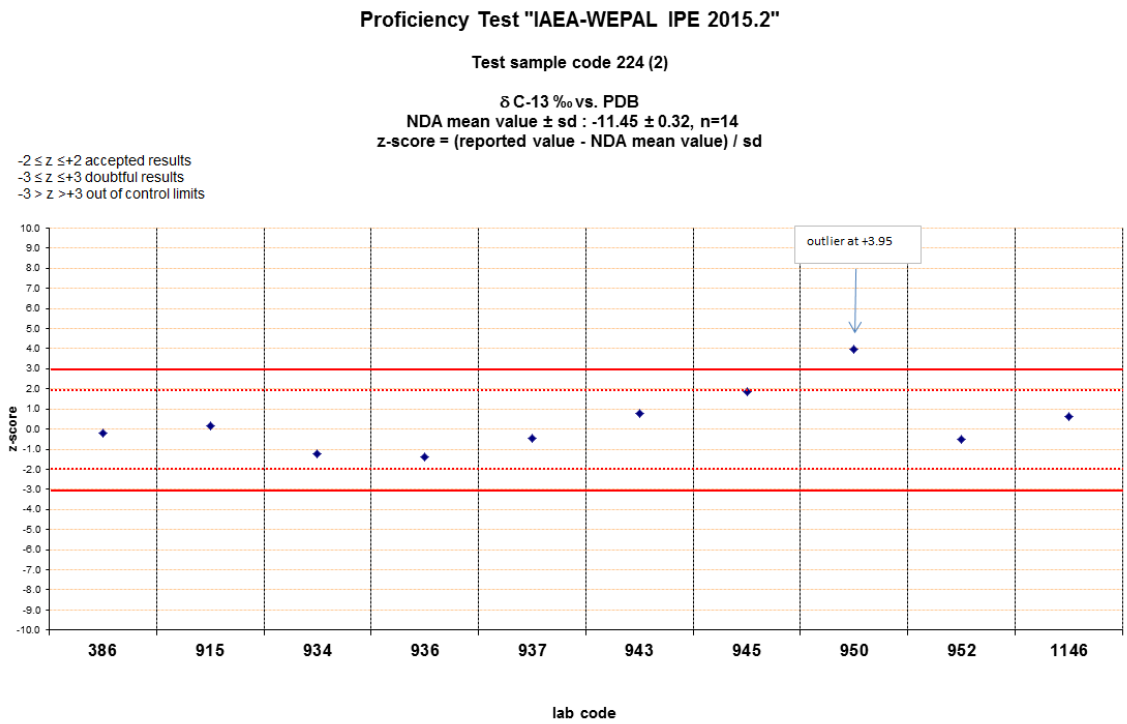


FIG. 13: Z-score evaluation of the ^{13}C analysis in the 2015 proficiency test.

GUIDELINES AND INFORMATION PUBLISHED IN 2015

Supporting sampling and sample preparation tools for isotope and nuclear analysis

This FAO/IAEA publication (IAEA-TECDOC-1783) was developed to provide illustrated, step by step, comprehensive guidance for sampling and processing of soil, water and plant materials. It aims to assists scientists, technicians and students in Member States in implementing procedures and tools to take and prepare samples for isotope and nuclear analysis in their efforts to develop climate-smart agricultural practices for improved soil, water and nutrient management and to prepare and respond to nuclear emergencies in food and agriculture. The TECDOC includes the following modules: (i) Particulate organic matter separation, (ii) Method for the purification of inorganic phosphate in soil and sediment samples prior to analysis of the $\delta^{18}O$ isotopic abundance in phosphate, (iii) Extraction of water from soil and plant samples for $^{18}O/^{16}O$ and D/H isotope ratio measurements, (iv) How to perform precise soil and sediment sampling? One solution: The Fine Increment Soil Collector and (v) Guidelines for measuring bulk density of soil. It can be found at: http://www-pub.iaea.org/MTCD/Publications/PDF/TE-1783_web.pdf.

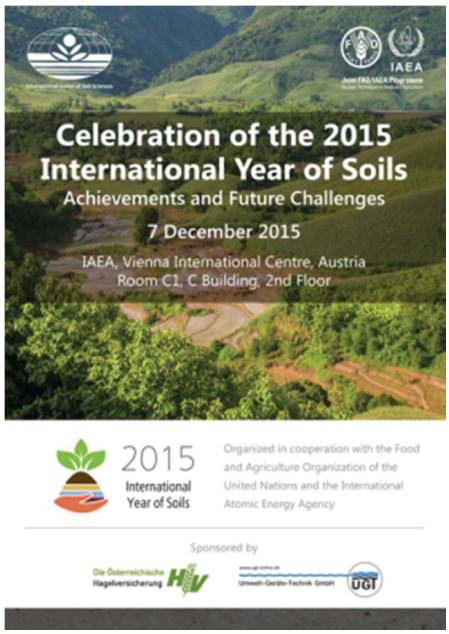
Animated FAO/IAEA infographic on ^{137}Cs for assessing and mitigating soil erosion

A new animated infographic video, aimed at the educated lay person, highlights the use of FRN techniques (^{137}Cs) as a soil tracer to investigate soil erosion and sedimentation processes in agricultural environments was developed. It can be found at: http://www-naweb.iaea.org/nafa/resources-nafa/soil-Erosion-stream32.mp4.

National Geographic explores Planet Earth: By the Numbers – Saving Soil

The work of the SWMCNL and the SWMCN Section on the use of fallout radionuclides (FRNs) and compound-specific stable isotopes (CSSI) to estimate soil erosion and sedimentation rates and to identify areas prone to erosion at the landscape level was published in the December 2015 issue of National Geographic magazine. The publication highlights how nuclear techniques can help in endeavours to assess and curtail the worldwide threat of soil erosion.

http://dl.yazdanpress.com/MAGAZINES/DOCUMENTARY/NATIONALGEOGRAPHIC/NATIONAL_GEOGRAPHIC_DECEMBER_2015.pdf.



Vienna Soil Declaration in the frame of the Celebration of the 2015 International Year of Soils: Achievements and Future Challenges, 7 December 2015, Vienna, Austria

More than 120 soil scientists gathered at the IAEA in Vienna, Austria to celebrate the "2015 International Year of Soils" and to highlight achievements and future challenges in soil science. The conference was organized jointly by the IAEA and the International Union of Soil Science (IUSS) and attended by representatives from the Food and Agriculture Organization of the United Nations (FAO), the European Commission through the Joint Research Centre (JRC), the Consultative Group of International Agricultural Research (CGIAR) and the President and the Regional Representatives of the IUSS.

At the event, the participants proclaimed the 'Vienna Soil Declaration: Soil matters for humans and ecosystems', which sets the framework for future research in soil science and links achievements to the United Nations' Sustainable Development Goals and global endeavours to combat climate change. It sends a strong message for The Future We Want. The Vienna Soil Declaration can be downloaded at http://www-naweb.iaea.org/nafa/swmn/Vienna-Soil-Declaration-Dec6-2015.pdf.

Success stories in the frame of the 2015 International Year of Soils

As part of the 2015 International Year of Soils, ten success stories were published by the SWMCN Subprogramme highlighting examples of country impacts derived through improving soil management across Africa, Asia and Latin America. Two stories were prepared by the SWMCNL team, i.e. (i) Sustainable agriculture of salt-affected soils in the lower Mesopotamian Plain of Iraq and (ii) Reducing soil erosion in Morocco. These stories can be downloaded at: http://www-naweb.iaea.org/nafa/swmn/iraq.pdf and at http://www-naweb.iaea.org/nafa/swmn/morocco.pdf

SWMCNL contributions at the European Geosciences Union (EGU) General Assembly 2015, Vienna, Austria

The European Geosciences Union (EGU) General Assembly 2015, held in Vienna from 12-17 April 2015, was attended by 11837 scientists attending from 108 different countries with 4870 oral, 8489 poster and 705 PICO (Presenting Interactive COntent™) presentations. As defined by the EGU organisers, PICO is bringing the advantages of both oral and poster together into an innovative type of presentation which opens the opportunity to be interactive. The activities of the SWMCNL were represented with three PICO presentations focusing on the combined use of FRN and CSSI techniques and one poster presentation (see list of publications below).

PUBLICATIONS

ALEWELL, C., BIRKHOLZ, A., MEUSBURGER, K., SCHINDLER WILDHABER, Y., MABIT, L. (2015). Sediment source attribution from multiple land use systems with CSIA. *Biogeosciences Discuss.* **12**: 14245–14269.

ARATA, L., LA SPADA, C., MEUSBURGER, K., ZEHRINGER, M., MABIT, L., ALEWELL, C. (2015). The ^{137}Cs repeated sampling approach to derive soil redistribution rates and validate reference sites in alpine grasslands. ENVIRA2015 International Conference on Environmental Radioactivity: New Challenges with New Technologies. 21–25 September, Thessaloniki, Greece.

ASARE, D.K., ANTHONIO, C.K., HENG, L.K., AYEH, E.O. (2015). Nodulation and fixed atmospheric nitrogen of some local lima bean (Phaseolus lunatus L.) cultivars grown in a costal savannah environment. Agricultural Sciences **6**: 925-933.

BENMANSOUR, M., MABIT, L., ZOUAGUI, A., AMENZOU, N., SABIR, M., NOUIRA, A., BRANDT, C., RASCHE, F., NAIMI, M., CHIKHAOUI, M., MARAH, H., BENKDAD, A., TAOUS, F. (2015). Combined use of fallout radionuclides and stable isotopes for investigating soil erosion processes in a Moroccan watershed. Geophysical Research Abstracts **17**: EGU2015-14793-2, EGU General Assembly 2015.

BENMANSOUR, M., YASSIN, M., MOUSSADEK, R., ZOUAGUI, A., NOUIRA, A., MABIT, L., HAJIB, S., MRABET, R., LAAICH, H., NDIATH, A.S. (2015). Role of Isotopic Techniques to Combat Land Degradation in Morocco. 12th session of the Conference of the Parties (COP 12) to the UN Convention to Combat Desertification (UNCCD). IAEA Side Event: Soil Science for Sustainable Land Management. 19 October 2015, Ankara, Turkey.

BIRKHOLZ, A., ALEWELL, C., MEUSBURGER, K., SCHINDLER-WILDHABER, Y., MABIT, L. (2015). From the soil to the river - CSIA of long-chain fatty acids as a fingerprinting tool for sediment source apportionment. PlantWax2015. 16–20 June 2015. Monte Verità, Switzerland.

BIRKHOLZ, A., ALEWELL, C., MEUSBURGER, K., SCHINDLER-WILDHABER, Y., MABIT, L. (2015). Tracking soil sediments to freshwater systems using CSIA of plant wax lipids. 5th International Symposium on Soil Organic Matter (SOM 2015). 20–24 September 2015. Goettingen, Germany.

BLAKE, W., TAYLOR, A., MABIT, L. (2015). Challenges and opportunities for use of natural fallout ^{7}Be as a soil erosion tracer in agricultural systems. Geophysical Research Abstracts **17**: EGU2015-10780, EGU General Assembly 2015.

DE CLERCQ, T., HEILING, M., DERCON, G., RESCH, C., AIGNER, M., MAYER, L., MAO, Y.L., ELSEN, A., STEIER, P., LEIFELD, J., MERCKX, R. (2015). Predicting soil organic matter stability in agricultural fields through carbon and nitrogen stable isotopes. Soil Biology and Biochemistry **88**: 29–38.

DE CLERCQ, T., XU, H., HEILING, M., DERCON, G., RESCH, C., MERCKX, R. (2015). Using old and new stable isotope techniques to evaluate the impact of conservation tillage on SOM dynamics and stability. 5th International Symposium on Soil Organic Matter (SOM 2015). 20–24 September 2015, Goettingen, Germany (abstract).

DE LOS SANTOS-VILLALOBOS, S., BRAVO-LINARES, C., ANJOS, R., CARDOSO, R., GIBBS, M., SWALES, A., MABIT, L., DERCON, G. (2015). A new user-friendly tool to assess soil redistribution using compound specific stable isotopes: The CSSIAR v1.00 Software. XVIII International Congress of Agricultural Sciences. 29-30 October 2015, Mexicali, Mexico.

HOOD-NOWOTNY, R. AND ALTER-NET MSII PARTICIPANTS (2015). Can isotopic signatures reveal reactive nitrogen priming of soil organic matter decomposition? In: Geophysical Research Abstracts **17**: EGU2015-7443, EGU General Assembly 2015.

IURIAN, A.R., DERCON, G., ADU-GYAMFI, J., MABIT, L., KIS-BENEDEK, G., CECCATELLI, A., TARJAN, S., BLAKE, W. (2015). The interception and wash-off fraction of ^{7}Be by bean plants in the context of its use as a soil radiotracer. *Journal of Radioanalytical and Nuclear Chemistry* **306**: 301–308.

IURIAN, A.R, OLUFEMI PHANEUF, M., MABIT, L. (2015). Mobility and Bioavailability of Radionuclides in Soils. In: Radionuclides in the Environment: Influence of chemical speciation and plant uptake on radionuclide migration (WALTHER, C., GUPTA, D.K., Eds), Springer, pp. 37–59.

KOUELO ALLADASSI, F., HOUNGNANDAN, P., AZONTONDE, H.A., BENMANSOUR, M., RABESIRANANA, N., MABIT, L. (2015). Assessment of the level of soil degradation in three watersheds affected by intensive farming practices in Benin. *Journal of Experimental Biology and Agricultural Sciences* **3**(6): 529–540.

LEIFELD, J., HEILING, M., HAJDAS, I. (2015). Age and thermal stability of particulate organic matter fractions indicate the presence of black carbon in soil. Radiocarbon **57**: 99-107.

MABIT, L., GIBBS, M., CHEN, X., MEUSBURGER, K., TOLOZA, A., RESCH, C., KLIK, A., EDER, A., STRAUSS, P., ALEWELL, C. (2015). Preliminary use of compound-specific stable isotope (CSSI) technique to identify and apportion sediment origin in a small Austrian catchment. In: Geophysical Research Abstracts **17**: EGU2015-11021-4, EGU General Assembly 2015.

RABESIRANANA, N., RALALARIMANANA, H., MABIT, L. (2015). Madagascar national programme to combat desertification and the support of isotopic techniques. 12th session of the Conference of the Parties (COP 12) to the UN Convention to Combat Desertification (UNCCD). IAEA Side Event: Soil Science for Sustainable Land Management. 19 October 2015, Ankara, Turkey.

WAHBI, A., VREUGDENHIL, M., WELTIN, G., HENG, L., OISMUELLER, M., STRAUSS, P., DERCON, G. (2015). Cosmic ray neutron probe, uses, calibration and validation in Austria. IAEA International Symposium on Isotope Hydrology: Revisiting Foundations and Exploring Frontiers. 11–15 May 2015, Vienna, Austria.

ZAMAN, M., MABIT, L., HENG, L. (2015). Land degradation and the use of nuclear techniques. International Soil Conference (ISC) on Sustainable Uses of Soil in Harmony with Food Security. 17–20 August 2015. Cha Am, Thailand.

ZAPATA, F., ZAMAN, M., NGUYEN, M.L., HENG, L.K., SAKADEVAN, K., DERCON, G., MABIT L. (2015). Innovations in soil and water management/conservation research through integrated approaches of nuclear and isotopic techniques and precision agriculture. In: Soil Specific Farming – Precision Agriculture, Advances in Soil Science (LAL, R., STEWART, B.A., Eds), CRC Press, pp. 247–282.

EXTERNAL COLLABORATIONS AND PARTNERSHIPS

Institution	Topic
Arctic and Antarctic Research Institute, Russian Federation	Climate change impact assessment in fragile environments
Austrian Agency for Health and Food Safety (AGES), Grabenegg, Austria	Assessment of carbon distribution and soil organic carbon sequestration in mulch-based cropping systems by using isotopic techniques; Assessment of soil moisture availability under mulch-based cropping systems

Institution	Topic
Austrian Institute of Technology, Health and Environment Department, Austria	Development of protocols for the use of stable isotope techniques for greenhouse gas emission screening
Austrian Research Centre for Forests, Austria	Climate change impact assessment in fragile environments
Belgian Nuclear Research Centre, Institute for Environment, Health and Safety, Belgium	Nuclear emergency response in food and agriculture
Chengdu Institute of Mountain Hazards and Environments; Chinese Academy of Sciences, China	Climate change impact assessment in fragile environments
Agroscope, Climate / Air Pollution Group, Switzerland	Soil organic carbon dynamics, Soil Organic Carbon-14 dating
Centre National de l'Energie, des Sciences et de Techniques Nucléaires (CNESTEN), Division Water, Soil and Climate, Morocco	Fallout radionuclides to assess erosion and effectiveness of soil conservation strategies Nuclear Emergency Response in Food and Agriculture
Department of Atomic Energy, Section of Environmental Studies, India	Nuclear Emergency Response in Food and Agriculture
Eidgenössiche Technische Hochschule (ETH), Institute for Agricultural Sciences, Switzerland	Use of oxygen-18 isotopes in phosphate to trace phosphorous sources and cycling in soils
Federal Agency for Water Management, Institute for Land &Water Management Research, Austria	Validation of the use of cosmic-ray soil moisture neutron probe for area-wide agricultural water management
Fujian Agriculture and Forestry University, College of Resource and Environmental Science, , China	Assessment of carbon distribution and soil organic carbon sequestration in mulch-based cropping systems by using isotopic techniques
European Commission, Joint Research Centre, Italy	Nuclear Emergency Response in Food and Agriculture
Hydroinnova, USA	Validation of the use of cosmic-ray soil moisture neutron probe for area-wide agricultural water management
Institut National des Sciences et Techniques Nucléaires, Department of Nuclear Analyses and Techniques, Madagascar	Fallout radionuclides to assess erosion and effectiveness of soil conservation strategies

Institution	Topic
Institute of Geography; Russian Academy of Sciences, Russian Federation	Climate change impact assessment in fragile environments
Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, China	Nuclear Emergency Response in Food and Agriculture
International Institute for Tropical Agriculture (IITA), Nairobi, Kenya	Assessment of carbon distribution and soil organic carbon sequestration in mulch-based cropping systems by using isotopic techniques
Japan Atomic Energy Agency, Japan	Nuclear Emergency Response in Food and Agriculture
Justus-Liebig University Giessen, Institute for Plant Ecology, Germany	Training in greenhouse gas emission measurement
Liverpool John Moore University, Faculty of Education, Health and Community, UK	Climate change impact assessment in fragile environments
Lomonosov Moscow State University, Faculty of Geography, Russian Federation	Climate change impact assessment in fragile environments
Los Gatos Research, USA	Support in stable isotope analysis of greenhouse gases
National Agricultural Research Organization, Tohoku Agricultural Research Centre, Japan	Nuclear Emergency Response in Food and Agriculture
National Institute of Water and Atmospheric Research, New Zealand	Climate change impact assessment in fragile environments
National Park Hohe Tauern, National Park Administration, Austria	Climate change impact assessment in fragile environments
National University of Life and Environmental Sciences, Ukrainian Institute of Agricultural Radiology, Ukraine	Nuclear Emergency Response in Food and Agriculture
Picarro, USA	Training in greenhouse gas emission measurement
Russian Institute of Radiology and Agroecology, Russian Federation	Nuclear Emergency Response in Food and Agriculture
Spanish National Research Council – CSIC, Department of Geology, Spain	Climate change impact assessment in fragile environments

Institution	Topic
Technical University of Vienna, Centre for Water Resource Systems, Austria	Validation of the use of cosmic-ray soil moisture neutron probe for area-wide agricultural water management
Universidad Austral de Chile, Institute of Chemistry, Chile	Compound-specific stable isotopes to improving soil conservation strategies at landscape level; Climate change impact assessment in fragile environments;
University of Rio de Janerio, Biophysics and Biometry Department, Brazil	Climate change impact assessment in fragile environments
Universidade Federal Fluminense, Laboratory of Radioecology and Environmental Change, Brazil	Compound-specific stable isotopes to improving soil conservation strategies at landscape level; Climate change impact assessment in fragile environments;
University of Basel, Environmental Geosciences, Switzerland	Fallout radionuclides to assess erosion and effectiveness of soil conservation strategies
University of Cologne, Institute for Geology and Mineralogy, Germany	Climate change impact assessment in fragile environments
University of Exeter, College of Life and Environmental Sciences, Geography, UK	Fallout radionuclides to assess erosion and effectiveness of soil conservation strategies; Climate change impact assessment in fragile environments
University of Ghent, Isotope Bioscience Laboratory, Belgium	Climate change impact assessment in fragile environments
University of Gothenburg, Department of Biological and Environmental Sciences, Sweden	Climate change impact assessment in fragile environments
University of Graz, Department of Geography and Regional Science, Austria	Climate change impact assessment in fragile environments
University of Idaho, Department of Geography, USA	Climate change impact assessment in fragile environments
University of Hohenheim, Institute of Agricultural Sciences in the Tropics, Germany	Support in the use of Mid-Infrared Spectroscopy techniques for soil characterisation
University of Leuven, Faculty of Biosciences Engineering, Division of Soil and Water Management and Division of Mechatronics, Biostatistics and Sensors, Belgium	Assessment of carbon distribution and soil organic carbon sequestration in mulch-based cropping systems by using isotopic techniques; support in training on statistics and experimental design to staff of Joint FAO/IAEA Division

Institution	Topic
University of Natural Resources and Life Sciences (BOKU), Institute of Hydraulics and Rural Water Management, Austria	Assessment of carbon distribution and soil organic carbon storage in mulch-based cropping systems by using isotopic techniques
University of Nebraska-Lincoln, School of Natural Resources, USA	Validation of the use of cosmic-ray soil moisture neutron probe for area-wide agricultural water management
University of Plymouth, School of Geography, Earth and Environmental Sciences, UK	Fallout radionuclides to assess erosion and effectiveness of soil conservation strategies (special focus on beryllium-7)
University of San Luis, Environmental Studies Group, Argentina	Development and testing of isotope and nuclear techniques for sediment tracing
University of Tsukuba, Centre of Research in Isotopes and Environmental Dynamics, Japan	Nuclear emergency response in food and agriculture
University of Vienna, Faculty of Physics and Department of Terrestrial Ecosystem Research, Austria	Climate change impact assessment in fragile environments; Soil dating using carbon-14
University of Wageningen, Wageningen Evaluating Programs for Analytical Laboratories (WEPAL), the Netherlands	External quality assurance: Annual proficiency test on nitrogen-15 and carbon-13 isotopic abundance in plant materials
USDA-ARS, Conservation & Production Research Laboratory, USA	Climate change impact assessment in fragile environments

AN UPDATE ON THE RENUAL PROJECT FOR THE FAO/IAEA AGRICULTURE & BIOTECHNOLOGY LABORATORIES

Buildings and infrastructure design coming to a close

The process of developing the designs for ReNuAL's new buildings progressed well during 2015 and is now nearing completion. After the *conceptual designs* for new Insect Pest Control Laboratory (IPCL) and Flexible Modular Laboratory (FML) buildings, which will house, among other, the Food and Environmental Protection Laboratory (FEPL) and the Soil and Water Management and Crop Nutrition Laboratory (SWMCNL), were reviewed and validated by a panel of external experts in February, the *functional design* of these buildings and associated infrastructure was completed in September. The functional design will be used to procure construction services for the buildings to move the project into the buildings construction stage.

Director General Amano kicks off construction

On the first day of the IAEA's 59th General Conference, held from 14-18 September, IAEA Director General Yukiya Amano was joined by the co-chairs of the *Friends of ReNuAL* group, Ambassador Friedrich Däuble of Germany and Ambassador Tebogo Joseph Seokolo of South Africa, at an event to mark the start of the construction phase of ReNuAL. The preparation of the Seibersdorf site for construction of the new infrastructure and laboratory buildings began on 7 September. Deputy Director General Aldo Malavasi, head the IAEA's Department of Nuclear Sciences and Applications, served as Master of Ceremonies for the event, which saw the unveiling of scale models of the new Insect Pest Control Laboratory and the Flexible Modular Laboratory buildings.

Director General Amano, Ambassador Däuble (Germany) and Ambassador Seokolo (South Africa) unveil scale models of the new laboratories with Deputy Director General Malavasi alongside. (Photo: IAEA)

Mr Amano expressed his appreciation for the support to ReNuAL and encouraged Member States to provide further financial support to ensure that the project can be fully funded. "*The progress we have made so far would not have been possible without the generous support of IAEA Member States*", said Mr Amano. "*I call on all Member States to continue to support the IAEA Nuclear Applications Laboratories and the ReNuAL project*."

Bulldozers take over the greenfields at Seibersdorf

On 7 September, bulldozers took over the greenfields at the Seibersdorf laboratories to prepare the site for construction. In just two weeks, more than 300 truckloads of soil and debris were removed, transforming the field into a construction-ready site.

The first building to be constructed through ReNuAL will be the IPCL facility. This will alleviate current severe space constraints and will increase capacity for developing and transferring new and innovative methods for insect pest control through the sterile insect technique (SIT). It will also increase the capacity to develop area-wide integrated pest management strategies for additional major insect pests.

Upcoming signing of construction contract a milestone for ReNuAL

Following completion in September 2015 of the functional designs of the new buildings, the tender process for the ReNuAL construction contract began in early October. Bids were received in December, followed by an evaluation and negotiation process that is scheduled to be completed with the awarding of the construction contract in February 2016. This will represent a significant milestone in the project and will bring the planning phase of ReNuAL to completion.

Construction of the new electrical infrastructure is planned to start in April 2016. This will make electricity available on site for construction of the new laboratory buildings and remaining infrastructure. Construction of the new IPCL building is scheduled to start in June 2016 with construction of the FML to commence as shortly as possible thereafter. This has been determined to be the quickest and most cost-effective schedule for the project's implementation, though the schedule remains dependent on the availability of the outstanding extrabudgetary funds that are still required to fully fund ReNuAL.

Biosafety level 3 operations set to begin

In March 2015, the Austrian government announced at the IAEA's Board of Governors meeting that an agreement had been reached for the IAEA to access a biosafety level 3 laboratory (BSL3) recently built by the Austrian Agency for Health and Food Safety (AGES) in Mödling. The IAEA's Animal Health and Production Laboratory will have full and exclusive access to this facility to implement activities related to the control of transboundary animal and zoonotic diseases, including training, with operations scheduled to begin in August.

While these new capabilities will significantly enhance the IAEA's ability to support Member States in this area, the IAEA and Austrian authorities continue consultations over the possibility of constructing an IAEA-dedicated extension to the facility in the future. Also in the March meeting, the Austrian government announced the offer of a package of land, infrastructure and technical services it values at €2 million to support construction of such a facility.

The fundraising momentum continues

Throughout 2015 Member States have continued to show their strong support for ReNuAL, with several financial and in-kind contributions supporting the project. Previous donors announced new pledges, beginning in August with the United States of America pledging a further US $1.12 million. During the IAEA's 59th General Conference, the United States announced a further pledge of US $3 million, China pledged €2 million and Kuwait pledged US $1 million. A total of 21 Member States have to date pledged financial support towards the funding of the ReNuAL project.

Also during the General Conference, Member States approved the IAEA's 2016-2017 Programme and Budget, which includes €2.5 million in regular budget funds to ReNuAL during each of these two years. Once these funds are allocated, the total capital regular budget funds provided to ReNuAL will reach the initially planned €10.4 million. Combined with the extrabudgetary funds of €14 million raised to date, this brings total funds planned and contributed for ReNuAL to €24.5 million.

Nevertheless, a further €6.6 million are still required to fully fund ReNuAL and to ensure that building construction can proceed expeditious and in the most cost-efficient manner. The current schedule calls for construction of the IPCL building to commence in June 2016, while the scheduling for the FML building will depend on timely securing the necessary funding.