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Laboratories

Activities Report **2018**

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

Contents

THE ANIMAL PRODUCTION AND HEALTH LABORATORY

EXECUTIVE SUMMARY

The Animal Production and Health Laboratory (APHL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture assists Member States (MSs) improving the productivity of livestock and preventing and controlling animal infectious diseases and zoonoses. Nuclear and nuclear related techniques for serology, molecular biology and genetics are the main technologies applied in the APHL and transferred to Member States' laboratories to support animal production and health.

The early detection, prevention and control of transboundary animal and zoonotic diseases (TAZD) continue to be the main priorities for MSs. Indeed, the veterinary services and laboratories around the globe are continuously challenged by the spread of infectious diseases in animals and from animals to humans or by the emergence of new diseases, such as the Middle East Respiratory Syndrome (MERS), zoonotic animal influenza or Zika. The most recent outbreak examples are the two threatening animal virus infections, Peste des Petites Ruminants (PPR) and African Swine Fever (ASF). In June 2018, PPR emerged for the first time in Europe, with 7 outbreaks in Bulgaria. In August 2018, ASF virus (ASFV) was notified in China for the first time; within a relatively short time, it has spread throughout several Chinese provinces and is currently threatening neighbouring countries. As we draft this report, we learned that ASF has indeed spread over to Mongolia and Vietnam and ASFV-contaminated products were detected at border inspection controls in Japan, South Korea and Taiwan. Meanwhile, ASFV is spreading into new territories in Europe with cases in domestic pigs in the Balkan region and wild boarsin Central Europe and Belgium. It also continues to cause high mortalities in sub-Saharan Africa. Fighting such outbreaks and threats to food security and human health, at the regional and global levels, requires Research and development (R&D), technology transfer to, and capacity building in MSs. That is what APHL is fostering to assist MSs in outbreak investigations and emergency response to diseases.

In 2018, APHL continued its work on three major animal health topics: 1) application of nuclear and nuclear derived technology for the development and evaluation of safer, more effective vaccines; 2) development and validation of laboratory diagnostic assays for the rapid and accurate detection of microorganisms causing animal diseases; and 3) assistance to MSs veterinary diagnostic laboratories for preparedness and rapid response to TAZD. This is done through technical assistance such as: scientific investigations on the molecular epidemiology and spread of the infections, and preparation/distribution of harmonized protocols, laboratory reference material and reagents.

Vaccines are key tools in the prevention and control of animal infectious diseases. The APHL is conducting research on the application of irradiation technologies (gamma-ray and e-beam technology) to produce more effective and safer vaccine candidates. As described in the first two chapters of this report, irradiation can also be applied to synthetic molecules and chemical compounds such as liposomes or trehalose to improve vaccine formulations and therefore vaccine quality and efficacy. In this regard, the use of molecular epidemiology pointed out the need to re-evaluate existing vaccine and control strategies to better control diseases. From genetic and molecular epidemiology investigations carried out in 2018 we learned that: (i) avian pox virus outbreaks in Mozambique between 2016 and 2018 showed the presence of 3 distinct but co-circulating virus lineages and highlighted the need to re-evaluate vaccine and control strategies for this disease in the country; (ii) the the PPR virus emerged in Georgia in 2016 had unexpected similarities with PPRV strains circulating in East Africa, suggesting yet undiscovered mechanisms of virus spread in the region; (iii) studied ASF in Africa gave evidence of the transboundary virus spread in West Africa pointing out the need for a trans-national approach for its control, revealing the potential silent circulation of certain ASFV genotypes in East Africa; and (iv) the detailed characterisation of Lumpy Skin Disease (LSD) virus isolates, in East Africa, helped understanding the movement of the virus and its evolution, enabling the implementation of more efficient control measures and diagnostic procedures.

In terms of early detection and rapid confirmation of the identity of the pathogen, the APHL continued to work on the development, validation and transfer of nuclear derived serological and molecular techniques for disease surveillance and early pathogens detection. The application of a multiplex diagnostic protocol developed in APHL and the subsequent confirmatory tests run in Seibersdorf enabled the veterinary laboratory in Zambia to diagnose pseudocowpox virus infections in cattle for the first time in the country. In 2018, APHL conducted the preliminary validation of rapid serological assay for the detection of antibodies against capripox viruses in cattle, sheep and goats, an important step towards the high throughput screening of capripox-susceptible population.

Under animal genetics, APHL made significant progress in planned R&D activities, particularly in establishing workflow for genome-wide typing and bioinformatics data analysis to support implementation of genomic solutions for improved animal breeding in MSs. Among APHL's contributions in 2018 were: (i) the completion of the genotyping of 5000RAD camel radiation hybrid panel to develop the first-generation radiation hybrid map for chromosome 16, and (ii) the establishment of a new protocol was established for DNA based evaluation and characterization of backyard chicken in Africa. Furthermore, significant achievements were made towards successful implementation of National Action Plans on Animal Genetic Resources in various MSs: with APHL support and through IAEA technical cooperation projects, (a) Bulgaria completed molecular characterization of three cattle populations, and (b) India developed baseline genetic information on 14 indigenous cattle breeds. In addition, APHL actively supported the establishment or strengthening of molecular genetic laboratories in various countries through provision of necessary equipment and laboratory supplies. These efforts were recognized and highly appreciated by many member states, most notably by Argentina, Bulgaria and Burkina Faso.

In addition to R&D, APHL made important contributions to capacity building, for the benefits of MSs, training course and workshops. A regional training course titled ""Genetics of Parasite Resistance in Sheep and Goats: Application of Genomics and DNA Marker Information to Improve Small Ruminant Breeding" was conducted at APHL premises in Seibersdorf. This training course was designed to help national efforts to establish sheep breeding program for improving host resistance against parasites in the Latin American countries. Two other training courses were organized in Seibersdorf for African and Asian MSs to strengthen laboratory staff capacity to rapidly diagnose diseases in the laboratory. Furthermore, an advanced "train-the-trainer" course was organized by APHL to prepare the potential trainers in better fulfilling their task of training other veterinary laboratory scientists. It was developed in response to the increasing need for training in various regions in Africa and Asia demands the establishment of a pool of trainers with up-to-date knowledge on laboratory technologies and protocols. The course was very positively received, and more courses of this kind are foreseen. In addition, APHL hosted 12 fellows/interns/scientific visitors and undertook eight technical support missions in 10 MSs laboratories/institutions to support activities on animal health and production.

Knowledge dissemination and data sharing has been central to APHL activities, through: (a) APHL involvement and coordination of the VETLAB Network of national veterinary diagnostic laboratories, the membership of which counts 45 African and 19 Asian countries; (b) sharing of technical data and scientific information with MSs and scientific communities (19 publications in refereed high impact scientific journals, one chapter in a scientific book, and 8 oral/poster presentations in international conferences); and (c) resource mobilization to enhance capacity building and technology transfer, with financial support from USA and Japan (IAEA Peaceful Uses Initiative), from South Africa (African Renaissance Fund) and OPEC Funds for International Development.

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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Animal Health

Use of radiation technology to develop a new generation of vaccines for animal infectious diseases

Irradiated vaccine adjuvant liposomes augment both innate and cell mediated immune responses in vaccines

Vaccines are the most cost-effective method used to control infectious diseases through the activation of host adaptive immune responses. Effective vaccines are also required to activate the innate immune system of the host at the site of inoculation through antigen presenting cells (APC) such as dendritic cells (DC) which eventually activate the adaptive immune system. Often, inactivated vaccines such as irradiated vaccines need "vaccine adjuvants" to activate innate immune system. Liposomes are nano-scale molecules with at least one lipid bi-layer that functions as vaccine adjuvant. Monophosphoryl lipid A (MPLA) is a compound that is derived from lipopolysaccharides of the walls of gram-negative bacteria which is safe and extremely potent in activating the cells of the innate immune system. Therefore, liposomes containing MPLA are excellent vaccine adjuvants and have been used along with many vaccine formulations to increase their potency. Their adjuvant effect is produced not only by activating immune cells but also by binding to antigens and transporting antigens into APC to facilitate the antigen presenting process to induce a strong adaptive immune response. Filtration is the method used to sterilize liposomes during production, however, some liposomes containing MPLA that consist of saturated phospholipids are too large to be filtered. As a solution to this problem, gamma irradiation could be used for sterilization. In the past, several groups have tried using irradiation on different types of liposomes with variable success rates. APHL started a collaborative project with Polymun Scientific Immunbiologische Forschung GmbH on irradiating vaccine adjuvant liposomes. The goal of this project was to assess the feasibility of using irradiation to sterilize liposomes and then to assess their function as vaccine adjuvants. As a first step of this investigation, we assessed various irradiation protocols and their effect on the structure and primary function liposomes containing MPLA. Early results from these experiments suggest that irradiation (25 kGy) could indeed be used to sterilize MPLA-liposomes without affecting their chemical properties or primary functions such as antigen uptake. Encouraged by these results, we wanted to investigate further if such irradiated adjuvant MPLA liposomes could augment vaccine induced immunity. We have therefore developed an in-vitro monocyte derived (Mo)DC based assay to investigate vaccine induced immunity. This assay could be used as an alternative to animal studies to measure cell mediated immunity as a preliminary measure. We used the in-vitro MoDC assay to investigate the irradiated adjuvant MPLA liposomes. In the first phase of this experiment, we assayed the innate ability of MPLA liposomes to activate/ mature MoDC. We first generated MoDC from monocytes and then incubated with or without MPLA liposomes that were irradiated at 25 kGy either at room temperature or under frozen conditions or not irradiated (Fig 01). Following incubation, MoDC were assayed by flow cytometry to assess their activation/maturation state by investigating the expression of markers CD86 and MHC class II. The results of this experiment suggest that the ability to activate immune cells by MPLA liposomes is unaffected by irradiation at 25 kGy (Fig 02).

Next, we used diphtheria toxoid (DT) as an antigen and investigated if addition of MPLA liposomes could augment the cell mediated immune response as shown in the Fig 03. In this experiment, we explored irradiation of MPLA liposomes at room temperature (RT), under frozen conditions, and under frozen conditions with the addition of trehalose - a sugar compound. DT was either adsorbed with irradiated (under above mentioned conditions) MPLA liposomes or not prior to pulsing with MoDC. Pulsed MoDC were cultured with naïve lymphocytes before the activation of lymphocytes was measured by expression of CD25 on CD4 and CD8 lymphocytes. Results suggest that addition of MPLA liposomes does increase the cell mediated immunity. Interestingly, the activation of lymphocytes was

confined to CD8 lymphocytes (Fig 04). The irradiation of liposomes under various conditions did not affect the augmentation of immunity. Moreover, irradiation under frozen conditions slightly increased the level of immunity compared to non-irradiated liposomes. These results suggest that irradiated liposomes could be used to enhance vaccine-induced immunity and used along with irradiated vaccines to control or prevent transboundary animal diseases.
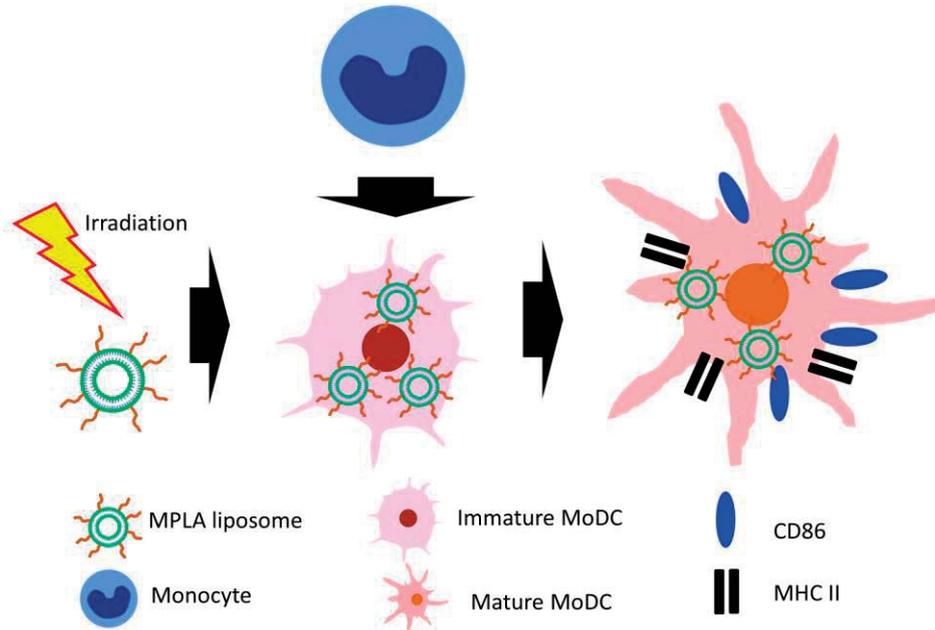
FIG. 01. Experimental protocol to investigate the effect of irradiation on MPLA liposomes on the maturation of MoDC. Monocytes were isolated from peripheral blood and then cultured with a cytokine cocktail to generate MoDC. MPLA liposomes were irradiated at 25 kGy either at room temperature or at frozen state. Then MoDCs were incubated with or without liposomes that were either irradiated or not.
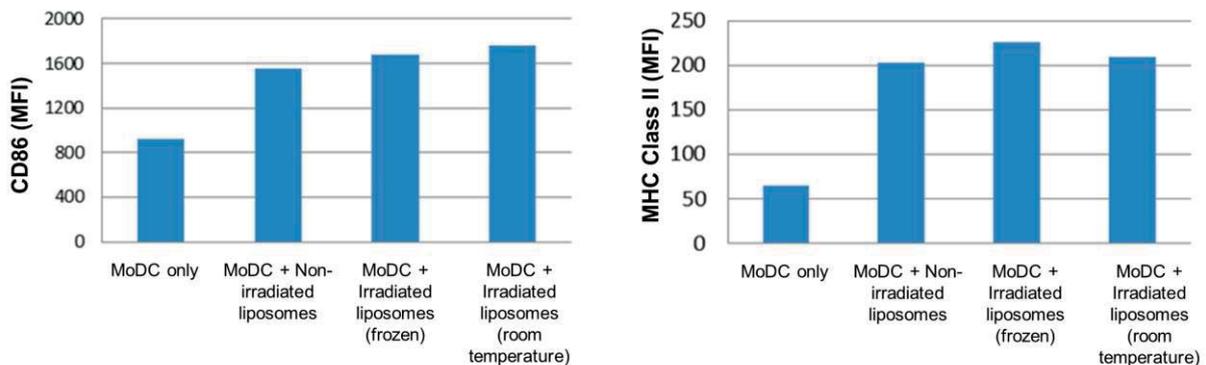
FIG. 02: Expression of maturation markers of bovine MoDC when incubated with irradiated liposomes. Following incubation, MoDC were harvested and stained for surface markers CD86 and MHC Class II. The cells were also stained for a live/ dead marker and assayed using flow cytometery. Mean fluorescence intensity (MFI) of CD86 and MHC Class II are shown.

Trehalose preserves antigenic proteins of virus vaccine candidates during irradiation

Gamma irradiation is a safe and effective method of inactivating pathogens to produce whole-organism vaccines. This method of vaccine development has been revitalised since the advent of newer irradiators that can deliver precise doses of radiation and allow a deeper understanding of the immune system. Because of the low cost of related R&D and comparatively faster time in their development process, irradiated vaccines are a good option for transboundary animal diseases for which effective vaccines do not exist. Already, in the market there is one irradiated vaccine for a nematode infection in cattle. The Joint FAO/IAEA Division of Nuclear Techniques in Food and

Agriculture is currently conducting a CRP with six Member States to develop irradiated vaccines for transboundary animal diseases. The APHL is providing technical improvements for these vaccine projects by developing improved protocols for irradiation and tools for assessing vaccine efficacy. In addition, APHL also conducts its own irradiated vaccine experiments in collaboration with other research institutes.

Irradiation · MoDC
MPLA liposome · CD8 T cells
Diphtheria toxoid · CD4 T cells
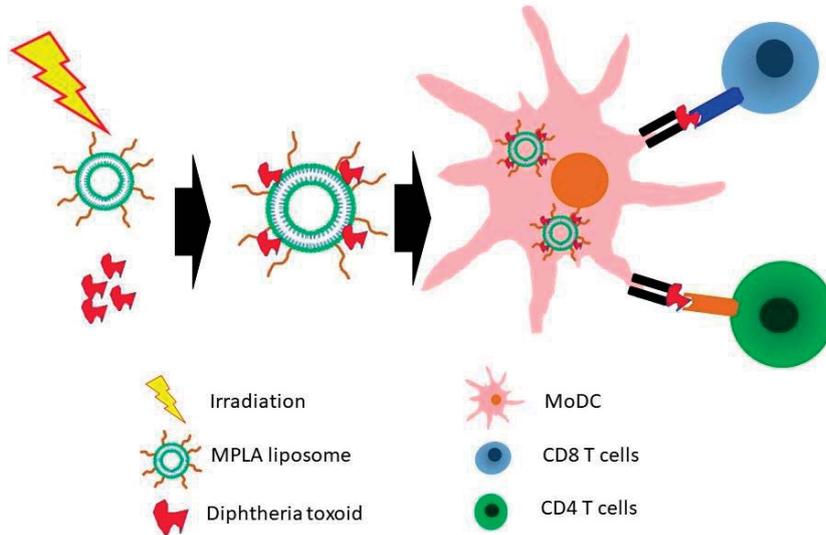
FIG. 03. Experimental protocol to investigate the effect of irradiation on MPLA liposomes in the augmentation of cell mediated immunity. Diphtheria toxoid (DT) was either adsorbed with irradiated (room temperature, frozen or frozen with trehalose) MPLA liposomes or not prior to pulsing with MoDC. Pulsed MoDC were cultured with naïve lymphocytes to investigate the activation.
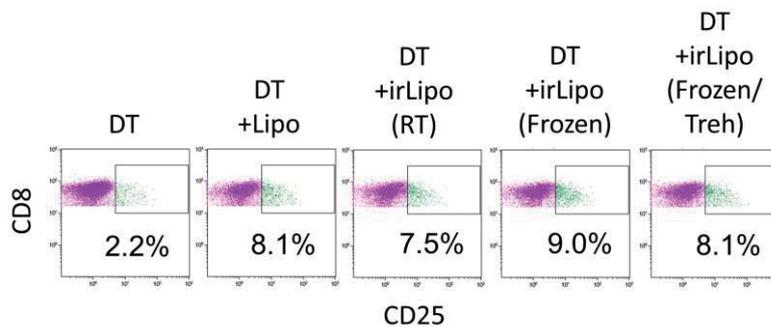
FIG. 04. Irradiated MPLA liposomes augment the cell mediated immunity similar to non-irradiated liposomes. Representative flow cytometry dot plots showing the CD25 expression by CD8 lymphocytes arising from cultures where MoDC were only pulsed with diphtheria toxoid (DT) or MoDC pulsed with DT adsorbed with non-irradiated liposomes [DT+Lipo] or irradiated at room temperature [DT+irLipo(RT)] or irradiated frozen with trehalose [DT+irLipo(Frozen)] or irradiated frozen [DT+irLipo(Frozen/ Treh)].

There are two types of irradiated vaccines: 1. Killed vaccines, a higher dose of radiation is used to kill the pathogens, 2. Metabolically active non-replicative vaccines: a lower dose of radiation is used only to inhibit the pathogen to stop virulence but to induce immunity. In both approaches, it is expected to preserve the epitopes that would induce a protective immunity in the host. However, especially with killed irradiated vaccines, the radiation dose could destroy the proteins (epitopes) of the pathogen that are responsible for immunity. It is believed such destruction is caused by free radicals that are produced during the irradiation process. Various irradiation protocols are currently in use to minimize such damage including irradiation under frozen conditions. Another approach has been the addition of radiation-protective compounds such as radio- protective Mn2+-Peptide complex from Deinococcus. But, synthesis of these peptides could be very expensive and commercial viability of such

vaccines remains low. Therefore, APHL investigated low cost methods to protect antigenic proteins during gamma irradiation. Two compounds were evaluated in these experiments: 1. Cerium dioxide nano-particles (CeO2) - proven for their highly anti-oxidant properties; 2. Trehalose (α-D-glucopyranosyl-(1→1)-α-D-glucopyranoside) - a sugar compound that is commonly used for vaccine stabilization. Bovine serum albumin (BSA) was used as the protein compound for the assay and it was irradiated at 25 kGy, at room temperature, with or without the addition of CeO2 or Trehalose. Following irradiation, BSA was visualized by bands resolved by protein gel electrophoresis (SDS-PAGE). The results suggest that Trehalose preserves proteins after irradiation at 25 kGy. Irradiation without protective compounds or addition of CeO2 destroyed almost all the BSA proteins (Fig 5 LEFT). We conducted further investigations on the use of Trehalose as a radiation-protective compounds in an irradiated vaccine against porcine reproductive and respiratory syndrome (PRRS). PRRS is a devastating viral disease present in swine globally that causes huge economic losses. Currently, there is a commercially available attenuated vaccine for the prevention of PRRS. However, this vaccine is not safe and not 100% effective. Therefore, there is bigger demand from the swine industry to develop safe and effective vaccine. APHL is currently conducting experiments with the Austrian Agency for Health and Food Safety (AGES) to develop an irradiated vaccine against the PRRS virus (PRRSv) infection in swine. The initial experiments proved the safety of the use of irradiated PRRSv as a vaccine candidate. In the subsequent experiments, the focus is on the development of irradiation protocols that would preserve the antigenicity of the virus during irradiation and produce a stronger immunity in the host. These experiments suggest that when Trehalose is used in the formulation, it does indeed preserve PRRSv proteins during irradiation (Fig 5 RIGHT). We will soon use irradiated PRRSv as a proto-type vaccine candidate and test its potential in the prevention of this disease in swine.
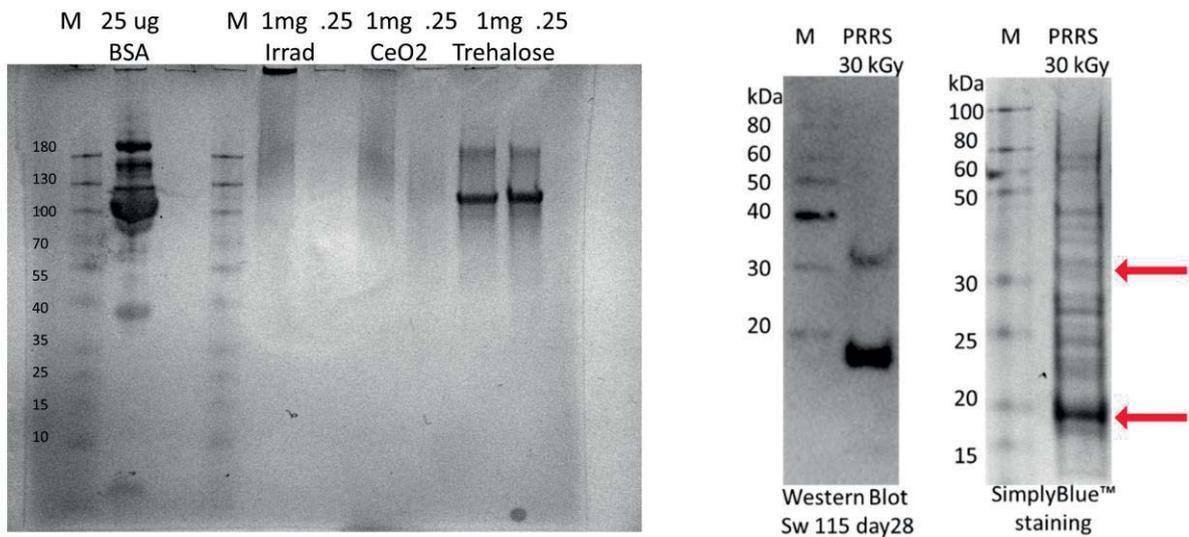
FIG. 5. *LEFT. Irradiation with trehalose protects BSA. BSA (1ug and 0.25 ug) was irradiated at room temperature at a dose of 25 kGy. Following irradiation BSA was visualized by bands resolved by protein gel electrophoresis (SDS-PAGE).M: marker.*

Figure 5: *RIGHT. Antigenic proteins are preserved following irradiation of PRRSv. 20 μl of concentrated PPRS virus in 25% Trehalose irradiated at 30kGy and subjected to western blotting using swine sera that revealed 2 bands at ~30 kDa and below 20 kDa, with the 20 kDa band at a higher intensity*

Tackling transboundary animal and zoonotic diseases

Tracing the emergence and spread of transboundary animal infectious diseases and understanding their epidemiology is extremely important to implement effective control measures and assist decision-makers during outbreaks. The APHL has been playing an active role to assist Member States in the development of specific and rapid diagnostic tests and the generation of genetic data used to characterize pathogens and to develop molecular epidemiological investigations. In this section we

report on the APHL activities in support of national laboratories to rapidly diagnose the disease and better understand the epidemiology of the infection.

Identification of Peste des Petits Ruminants, Georgia

The peste des petits ruminants virus (PPRV) is the cause of a highly infectious transboundary animal disease that primarily affects sheep, goats and small wild ruminants. It is presently being targeted by international organizations for global eradication by 2030.

Between January and March 2016, outbreaks of PPR were reported in three farms located near Tbilisi, the capital of Georgia. Organ and swab samples were collected and tested in the Laboratory of the Ministry of Agriculture, Tbilisi, using a PPR Antigen Capture ELISA. As epidemiological investigation could not establish the source of the infection in Georgia, six positive samples were then shipped to Austria in 2018 for further characterization and molecular epidemiological investigations by APHL. Amplicons were purified, sequenced and analysed. The phylogenetic analysis of N and F virus gene segments revealed that the PPRVs present in the three Georgian samples were identical and belong to lineage IV (Figure 6 A and B). Notably, the N gene fragment sequences were more related to those of viruses from Egypt, Eritrea, Ethiopia, and Sudan while the F gene fragment sequences clustered with viruses from Egypt, Ethiopia and Sudan. Unexpectedly, the N and F gene fragment sequences for viruses isolated from countries close to Georgia (e.g. Turkey, Iran and Iraq) were less similar to the Georgian viruses.

This is the first report of PPR in Georgia. Since there is no obvious connection between Georgia and Egypt, Eritrea, Ethiopia or Sudan through the trade or import of small ruminants, further work is required to fully understand PPRV circulation at a regional level.

Molecular characterization of Avipoxviruses circulating in Mozambique 2016-2018

Avipoxviruses (APV) are large enveloped DNA viruses that belong to the genus Avipoxvirus in the Chorodopoxvirinae subfamily of the family Poxviridae. APVs cause Fowlpox (FP) disease which can result in significant economic losses in domestic poultry (e.g. chicken and turkey) due to a decline of egg production, reduced growth, blindness and increased mortality which can reach 50%.

APV affected flocks between November 2016 and January 2018 in 18 separate outbreaks in four provinces (i.e. Inhambane, Maputo, Maputo city and Sofola) of the country. They were investigated by the Central Veterinary Laboratory (CVL), Maputo, in collaboration with APHL. The outbreaks primarily affected backyard chickens and egg layers, and at lesser scale, flocks of turkeys, peacocks and quail. All the samples yielded positive PCR results using APV specific primers. Representative amplicons were purified and were sent to LGC Genomics (Berlin, Germany) for sequencing through the service provided to Member States by the Joint FAO/IAEA Division.

The results for the genetic analysis using both the 4b core protein and DNA polymerase gene fragments from representative samples, from each flock, clearly showed that 16 of the samples analyzed grouped in Clade A1, previously reported in the country. The remaining three samples clustered in Clade A2 along with viruses identified in a variety of birds from South America, Europe and Asia. To date, Clade A2 APVs have been identified in wild Columbiforms (e.g. doves and pigeons), penguins, raptors, partridges, quail and great bustards but never in chickens or turkeys. This is, therefore, to the best of our knowledge, the first report of Clade A2 APVs in turkeys.

An official control program for FP in Mozambique does not exist and because the country does not have a poultry production system that meets the national demand, poultry and poultry products are often imported from Brazil and neighbouring countries, such as South Africa, Swaziland, and Zimbabwe. Of the poultry investigated in the present study, none of the backyard flocks or broilers were vaccinated against FP while the turkeys were vaccinated with a live vaccine. The egg layers were purchased already vaccinated mainly from South Africa where a live FP-vectored Mycoplasma gallisepticum (MG) vaccine in combination with classic live Avian encephalomyelitis (AE) virus is used.

Therefore, the occurrence of FP in both vaccinated and un-vaccinated poultry caused by three genetically distinct APVs (i.e. Clade A1, Clade A2 and Clade E – previous study) requires urgent re-evaluation of the vaccine and control strategies used for FP in Mozambique.
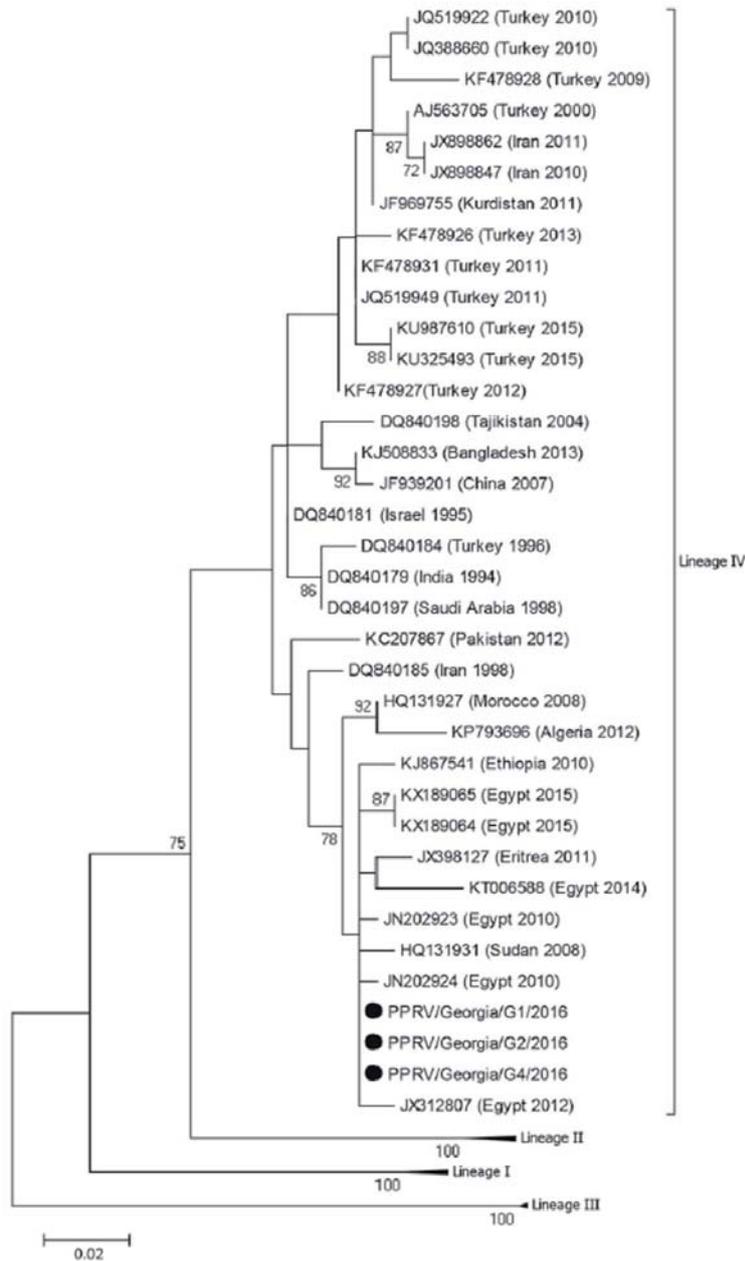
FIG. 6: Maximum likelihood tree of the PPR virus N gene segments. The isolates of this study are highlighted with black circles.

Molecular epidemiology of African swine fever (ASF)

ASF is a highly lethal haemorrhagic disease of domestic and wild swine, endemic in several African countries, and emerging in Europe and Asia.

Since 2012, APHL is supporting the efforts of several African Member States to tackle ASF by reinforcing the local laboratory capacities to detect and perform an in-depth analysis of the ASF virus. Recently, as of 2017, APHL has been working with VETLAB partners in Burkina Faso, Cameroon Cote d'Ivoire, Mozambique and Tanzania to further characterise their local ASFV isolates collected during 2016 to 2018 outbreaks period to better understand the epidemiology of the disease.

All isolates collected in Cameroon between 2010 and 2018 were classified as ASFV genotype I based on the partial analysis of B646L gene (C- terminal end of VP72 gene) and the full analysis of E183L gene encoding p54 protein. Furthermore, analysis of the central variable region (CVR) within the B602L gene demonstrated that there were 3 different variants of ASFV genotype I, with 19, 20, and 21 tetrameric tandem repeat sequences (TRSs), that were involved in the 2010–2018 outbreaks. Among these, one variant with 19 TRSs was identical to the Cam/82 isolate found in the country during the first outbreaks in 1981–1982. The three variants of ASFV isolates identified in these outbreaks were similar to those detected in neighbouring countries, suggesting a movement of ASFV strains across borders. These findings which are useful for designing common regional control measures were disseminated through a peer-reviewed paper (Journal of Microbiology, In Press, DOI 10.1007/s12275-019-8457-4).

Similarly, APHL and its partners in Ivory Coast characterized ASFV collected during the 2014 outbreaks in the country. Phylogenetic analyses based on p72 and p54 sequences showed that the San-Pedro 2014 outbreak virus strain belongs to p72 genotype I. These findings were disseminated through a peer-reviewed paper (Transboundary and Emerging Diseases, In Press, DOI: 10.1111/tbed.13098). Likewise, in Burkina Faso, only genotype I AFSV was identified in samples form outbreaks in 2014, 2016 and 2018.

In contrast, genotype II was involved in outbreaks in Mozambique (2016 and 2017) and Tanzania (2015 and 2016). Interestingly, in Tanzania, samples collected from outbreaks with high mortality and morbidity contained only genotype II isolates, while only genotype IX was associated with outbreaks with low morbidity and mortality, indicating that at least two genotypes of ASFV are circulating in the country. This highlights the risk of underestimating the presence of genotype IX because of its related low morbidity and mortality. Therefore, continuous monitoring of outbreaks and characterisation of local isolates remain a high priority.

Molecular epidemiology of Lumpy skin disease in Eastern Africa

Lumpy skin disease (LSD) is an important transboundary disease of cattle. Initially confined to the Africa continent, LSD has moved as of 2010 to the Middle East where it became endemic and has emerged in Europe in 2015. APHL supports MSs in the control of LSD by developing diagnostic tools (serological and molecular), molecular epidemiology tools and improving vaccines against LSD. The continuous characterisation of LSD isolates can assist in understanding the movement of the virus as well as its evolution, thus enabling the implementation of more efficient control measures. APHL has worked with Members States partners in Ethiopia, Sudan and Kenya to characterised LSDV isolated from outbreaks that occurred between 2011 and 2015 and compared them to LSDVs samples collected earlier in the same region and elsewhere. The analysis of the GPCR and the RPO30 genes showed that LSDVs were in general well-conserved. However, an isolate from Kenya featured, on the GPCR gene, a 12-nucleotide insertion, similar to the one observed in LSDV vaccine strains like KS1 and Neethling. A close look at all the publicly available GPCR sequences of field isolates shows that all the isolate B338/2011 from Kenya differ from all recent field isolates of LSDVs. Similar profiles were found only in two historical isolates: the LSDV RSA/54-Haden isolated in South Africa in 1954 and the LSDV NI-2490-Neethling-2490 isolated from cattle in Kenya in 1958. Such isolate may be difficult to differentiate from the most commonly used vaccine strains (KS1 and Neethling) using PCR and real time PCR method. However, a close look at the GPCR, the RPO30 and the EEV glycoprotein gene sequences, displayed some distinct features from the KS1 and Neethling vaccine strain. This demonstrates the need for continuous monitoring of the genetic of LSDV isolates to better refine the control strategies. Further work is being undertaken to sequence the full genome of the isolate B338/2011 to determine if this isolate could have resulted from a recombination event.

First report and characterisation of Pseudocowpox in Zambia

Pseudocowpox is a pox disease of cattle caused by Pseudocowpox virus (PCPV), member of genus parapoxvirus in the family Poxviridae. PCPV causes morbidity and loss of productivity in cattle and can

infect humans working in close contact with infected animals. Clinically, due to the similarity of the clinical signs to those of lumpy skin disease in cattle, PCPV infections are often misdiagnosed.

In December 2017, the Central Veterinary Research Institute (CVRI) in Zambia, received samples consisting of skin nodules on suspicion of lumpy skin disease infection. The samples were from cattle of a mixed dairy and beef herd located in the Central Province of Zambia. An HRM assay for the simultaneous detection and differentiation of poxviruses of medical and veterinary importance, developed at APHL and transferred to CVRI during a field mission in April 2018, was used to screen the suspected samples. The analyses revealed the presence of PCPV in the samples (Figure 7), which was confirmed on samples collected during a follow-up mission to the same farm. Furthermore, the sequencing of the full B2L gene and phylogenetic analysis of the samples established the presence of PCPVs, closely related, but distinct from publicly available PCPV B2L sequences.

This first report of PCPV virus in Zambia illustrates the usefulness of multiple pathogens detection for differential diagnosis of pox diseases.
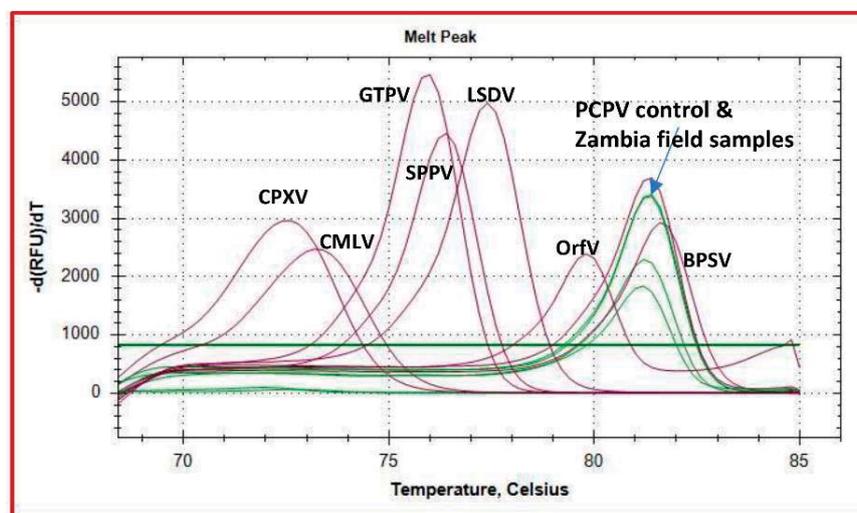
FIG. 7. *Melting curve analysis of the eight poxviruses standard and samples collected from cattle in Zambia. The Zambian field samples indicated in green colour are matching with pseudocowpox virus.*

Rapid differentiation of Sheep poxvirus vaccines from field isolates and other Capripoxvirus species

The genus Capripoxvirus (CaPV) within the family Poxviridae comprises three closely related viruses, sheep poxvirus (SPPV), goat poxvirus (GTPV) and lumpy skin disease virus (LSDV). Capripox diseases: sheep pox (SPP), goat pox (GTP) in sheep and goats and lumpy skin disease (LSD) in cattle are controlled mainly by using a live-attenuated vaccine. Although the appearance of the disease in previously vaccinated cattle herd is well documented, the problem in small ruminants is neglected. APHL has been working recently to develop tools to investigate SPP and GTP in previously vaccinated sheep and goat herds. We recently reported on the development of a new diagnostic tool for differentiation of SPPV field isolates from attenuated vaccine strains using gel-based PCR alone or in combination with sequencing (Chibssa et al. Virology Journal (2018) 15:59 https://doi.org/10.1186/s12985-018-0969-8). This work was further extended to develop an HRM-based method to differentiate SPPV vaccines from SPPV field isolates and further enable the genotyping of capripoxvirus isolates into SPPV, GTPV and LSDV. Sixty-one (61) capripoxvirus samples of various genotypes and geographical origins were tested using the HRM-based method. The assay has correctly classified 4 SPPV vaccines, 14 SPPV field isolates, 11 GTPVs and 32 LSDVs. The HRM-based results were confirmed by gel-based PCR method and sequencing data. These methods are being suited for routine use during outbreak investigations in both capripoxvirus enzootic and disease-free countries.

Evaluation of an Indirect ELISA for Detection of Capripoxvirus Antibodies in Goat, Sheep and Cattle Sera

Lumpy Skin Disease (LSD), Sheeppox (SPP) and Goatpox (GTP) are contagious diseases of ruminants with a devastating impact on the livestock industry and trade. LSD, SPP, GTP were mainly confined to Africa, the Middle East and Asia, with some sporadic incursions of SPP in Greece and Bulgaria. However, in 2015 the first incursions of LSD occurred in the European Union. Due to their potential for rapid spreading, a highly sensitive and specific serological method is needed for the surveillance of SPP, GTP and LSD and post-vaccination monitoring.

During 2018, the Animal Production and Health Laboratory worked on the expression, purification and evaluation of CVSP, a surface protein of the capripox virion as a candidate antigen for the development of an iELISA for detection of anti-capripoxvirus antibodies. The iELISA, in its present form, has been tested in more than 1,500 positive and negative sera samples. The test proved to be sensitive and specific. Additional validation tests and statistical calculations are currently on going and a publication is expected in the coming months.

Animal Genetics

Radiation hybrid mapping for dromedary camel

Genomic resources are scarcely available for genetic improvement of dromedary camel, an important livestock for nomadic pastoralists in Africa and Asia. During 2017, APHL announced the development of two radiation hybrid panels for dromedary camel, 5000RAD and 15000RAD. In 2018, APHL continued the characterization of camel radiation hybrid panel to develop the first-generation camel radiation hybrid map targeting chromosome 16. A total of 192 novel markers were designed, of which genotyping conditions were optimized for 150 markers. 5000RAD panel was typed for these novel DNA markers using conventional polymerase chain reaction (PCR) and integrated fluidic circuits based real time PCR methodologies. APHL in collaboration with International Camel Genome Consortium also initiated whole genome survey sequencing of camel radiation hybrid panels. The data will be used to construct whole genome map and reference genome assembly to facilitate the development of DNA based tools for breeding and improvement of camels.

Development of genetic tools for marker assisted dairy cattle improvement

Dairy production in tropics is characterized by low input system based on crop residues, agro-industrial by-products, community grazing, etc. and productivity per cow (milk yield/day/cow) is relatively low. The reliable and sustainable supply of improved animal genetics is one of the major obstacles for increased productivity. Developing countries in South Asia and Sub-Saharan Africa with small holder production systems lack organized breeding structure for dairy cattle (except for few institutional capacities). Genetic improvement of dairy cattle has been attempted mostly through crossbreeding (by AI using semen from intensely selected temperate breeds like Holstein-Friesian, etc.) rather than selection of superior indigenous animals. However, crossbreeding programs were constrained by several factors resulting in lack of stabilization of crossbreds with desired genetic makeup. This led to varying levels of genetic admixture in crossbred animals with problems of adaptability, reproduction, etc. Survival and performance of crossbreds under field conditions did not improve to the expected level due to various factors related to genotype X environment interaction. The fundamental limitation is the absence of infrastructural facilities for pedigree and performance recording and lack of efficient genetic evaluation programs to meet out the demands for genetically superior breeding bulls. Further, the genetic composition of crossbreds available with the farmers is not known and the performance of the crossbred cattle is not optimized for the existing production environment.

To address the issue, APHL initiated the development of low cost genomic tools for genotype detection in crossbred cattle. As a first step, baseline genetic differentiation of Asian Zebu, European taurine cattle and their crossbreds was established using classical microsatellite and genome-wide markers. A

total of 19,872 genotype data were generated from 736 cattle at 27 short tandem repeat marker loci. Bayesian structure analysis without prior population information clearly established the zebu-taurine divide and also indicated the level of genetic purity among zebu cattle breeds (Figure 8). Based on these results, more than 350 purebred zebu cattle were identified and typed for genome-wide single nucleotide polymorphic markers along with 54 purebred taurine and 48 crossbred cattle.
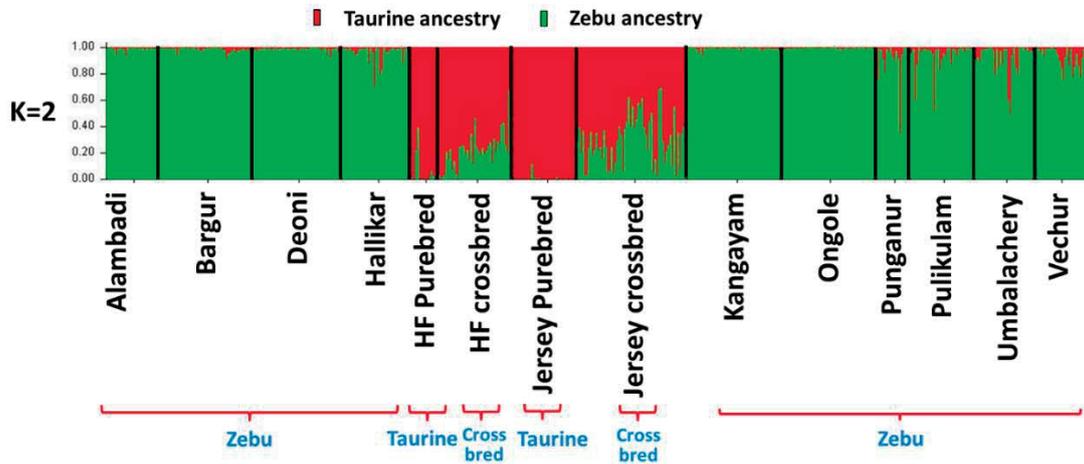

FIG. 8. *Deriving ancestry information to determine genetic admixture levels in indigenous zebu and crossbred cattle from Asia*

A custom designed pipeline for bioinformatics analysis of genomic data was established to perform biodiversity and genetic admixture analysis. A sub-set of SNP markers to design a low-density panel was identified to estimate genetic composition in crossbred cattle. The marker panel needs further expansion to include parentage testing capability and validation in crossbred cattle under field settings.

Screening indigenous Asian goat breeds for genetic resistance/susceptibility to transmissible spongiform encephalopathy

Scrapie is a well-known disease of goats worldwide. Prion protein gene (PRNP) polymorphisms have been shown to strongly modulate the resistance/susceptibility to the disease. APHL collaborated with Friedrich-Loeffler-Institut, Institute of Novel and Emerging Infectious Diseases, Germany to screen indigenous Asian goat breeds for PRNP gene variations. More than 1,000 goats belonging to 16 different breeds/populations located in six Asian countries (India, Bangladesh, Sri Lanka, Myanmar, China and Pakistan) were investigated. A total of 13 polymorphisms were detected within PRNP, of which nine are associated with an amino acid exchange, while four were silent mutations. Among these, 11 have been reported earlier while variations at S135N and L141L are novel and reported for the first time. The widely reported Q222K and R211Q alleles that are strongly associated with disease resistance were not observed in any of the Asian goat breeds studied. However, N146S allele reported to confer Scrapie resistance through the productive lifetime of goats was found in relatively higher frequency among Chinese crossbred (8.3%) and Yichang White (5.8%) goats. Similarly, G127S allele was at least three times more prevalent in certain Chinese and Indian goat populations as compared to European goats. R154H allele was either less frequent or completely absent among goats in different countries indicating the potentially low risk of Asian goats to atypical scrapie. Haplotype reconstruction revealed a total of 14 unique PRNP haplotypes with the observed number ranging from 5 (Myanmar and Pakistan) to 10 (Sri Lanka) across goats from different countries. Three major central PRNP haplotypes were observed among Asian goats as compared to two such haplotypes found in North American and European goats. Prevalence of the third major central haplotype originating from H143R dimorphism was distinctly high among Asian goats. Haplotype based tests for selective neutrality using Tajima's D, Fu and Li's D and Li's D statistic did not reveal any significant deviation in any of the Asian goat breeds investigated. Further studies addressing the disease association of PRNP

polymorphisms in Asian goats are required to establish a possible explanation for the low prevalence/reporting of Scrapie cases in the region.
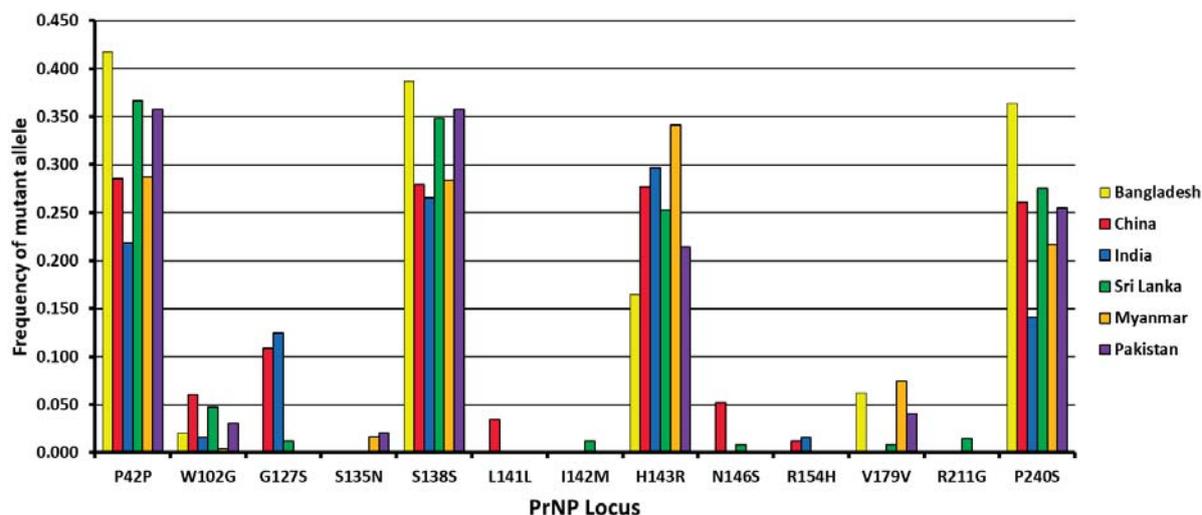

FIG. 9. Frequency distribution of 13 polymorphic (synonymous and non-synonymous) loci within PRNP gene in indigenous Asian goats.

Implementing Global Plan of Action for Animal Genetic Resources (AnGR)

In continuation of Joint FAO/IAEA efforts towards implementing Global Action Plan on Animal Genetic Resources (AnGR), APHL supported genetic characterization of 19 livestock breeds including 14 cattle breeds from India, 3 from Bulgaria and 2 from Burkina Faso. The results of molecular characterization will help to establish their population structure, genetic admixture levels and demographic dynamics. The genetic biodiversity information will be utilized in formulating effective strategies for the conservation and genetic improvement programs of the above-mentioned cattle populations. Further, APHL continued its efforts to improve the laboratory capacity of Member States for management of animal genetic resources. Institutional and technical support was provided to three countries (Burkina Faso, Cambodia and Nigeria) for establishing/strengthening molecular genetic laboratories through provision of necessary equipment and laboratory supplies under the framework of national technical cooperation projects.

New protocol for DNA based evaluation and characterization of backyard chicken in Africa

Backyard chicken play an important role in ensuring food security in rural areas of Africa. Developing baseline information on local chicken ecotypes is an essential step to understand and exploit the available genetic potential for better productivity. APHL designed and developed multiplex panels to genotype microsatellite markers recommended by FAO for biodiversity evaluation and characterization. A validated protocol of six novel marker panels containing 29 markers was developed and the manual is ready for transfer to Member States.

CAPACITY BUILDING

Interlaboratory Comparison (ring test) for peste des petits ruminants virus (PPRV)

In 2018, the APHL organized the yearly interlaboratory trial for the molecular and serological detection of PPRV. This exercise is a confidential, blind test of the ability of a diagnostic laboratory to determine the presence of PPRV in the samples provided. This year, the test panel contained 18 sample vials in total; 8 for nucleic acid and 10 for antibody detection in serum. All material was gamma-irradiated and, therefore, non-biohazardous. In total, 31 laboratories in 26 countries (22 in Africa, 7 in Asia, 2 in Europe) confirmed their participation. Based on the results of the ring test, the APHL is assisting participants in implementing corrective measures, when needed. APHL encourages PPR diagnostic

laboratories in Member States to make use of this annual exercise and test their ability to correctly detect PPRV.

Technical field support missions to build capacity in veterinary diagnostic laboratories

APHL has been actively involved in the transfer of technologies to Member States and support their activities to improve animal production and health. Several field support missions were undertaken to install and calibrate critical equipment, train local staff on animal genetics and reproduction, rapid diagnosis of animal and zoonotic diseases, improve diagnostic skill and animal vaccines production.

Technical visit to the national veterinary laboratories in Senegal and Mauritania

A mission was carried out in Senegal (Laboratoire National d'Elevage et de Recherches Vétérinaires Dakar) and Mauritania (Office National de Recherches et de Développement de l'Elevage – Nouakchott) aiming at evaluating the biosafety and biosecurity level of the national veterinary diagnostic laboratories and identifying areas for assistance and support to strengthen the laboratory capacities to detect and confirm zoonotic diseases relevant for the region. During the visit, the capabilities of these laboratories to handle and process samples potentially contaminated with dangerous zoonotic pathogens were assessed and biosafety reviewed. Furthermore, the mission identified technical and scientific activities focusing on major zoonotic diseases that could be rapidly implemented or strengthened within the VETLAB Network.

Field support mission to improve backyard poultry production in Burkina Faso

A field support mission to Burkina Faso was carried out by APHL staff to support the national efforts in improving backyard poultry production through improved genetics, breeding, nutrition and healthcare. Visit to the family poultry farms in Burkina Faso and discussion with local farmers revealed chick hatchability as one of the main challenges in backyard poultry. The hatchability of the local chicken and guinea fowl, ranging around 40% to 50%, can be increased up to 80% to 90% using specialized incubators. Access to incubators/hatchers for the local farmers was one of the serious constraints in improving backyard poultry production. Seven sites were identified for installation of pilot hatchery units to support the farmers and improve hatchability of local poultry. A technical program was set up to conduct a field survey on different poultry populations and locally available feed resources in Burkina Faso including medicinal plants. Information on morphology and DNA based genetic clustering will be used to classify local chicken populations into different genetic groups. Technical guidelines were provided for setting up a field trail to evaluate the production performance of different genetic groups. The trial results are expected to help the national experts recommend an optimal genotype for backyard poultry farming in Burkina Faso.

Technical mission to the Central Veterinary Research Institute (CVRI), Zambia

One APHL staff member travelled to Zambia to transfer and implement technologies related to the rapid multi-pathogen detection for the early diagnosis of Transboundary Animal Diseases (TADs) at CVRI. During this mission, a real time PCR platform was installed at the CVRI and twenty-one scientists of CVRI were trained in molecular detection of African swine fever virus (ASFV), highly pathogenic avian influenza virus (HPAI), peste des petits ruminants virus (PPRV), Mycoplasma mycoides mycoides, capripoxvirus (CaPV) and multi-parametric detection of pathogens causing respiratory diseases in small ruminants and pox-like diseases in ruminants and camels. This will enable the CVRI to expand its diagnostic capacities by including a number of real-time PCR-based assays. In addition, the laboratory staff was trained to undertake sequence analysis for a more complete and accurate characterisation of animal pathogens.

Technical mission to the Central Disease Investigation Laboratory (CDIL), Bangladesh

CDIL is one of the beneficiary laboratories of VETLAB network under the funding of the IAEA peaceful uses initiative (PUI) Asia. Under this project, APHL staff visited CDIL and supported five days training course on transfer of multiple pathogen detection assays. Thirteen participants attended the training,

which provided real time experience of handling nucleic acid-based diagnostics. The participants were also trained on sequencing work flow including sample preparation, sequence data handling and phylogenetic analysis. Now possessing real time PCR based PPR detection assays, CDIL expressed interest to include these in their routine screenings, participate in future PPR proficiency testing and in syndromic surveillance of respiratory disease in small ruminants, one of activities of PPR global eradication programme.

Technical visit to the national veterinary laboratories in Ghana and Cote d'Ivoire

The main objectives of the visit to the national veterinary laboratories in Accra and Abidjan were i) to assess the suitability (e.g. infrastructure, biosecurity, HR expertise) of the central veterinary laboratories in Cote d'Ivoire and Ghana to run molecular diagnostics for priority animal and zoonotic diseases and ii) to identify gaps and needs for guiding future technical assistance (e.g. needs for training and laboratory equipment). Given the expertise available in these national veterinary laboratories, members of the VETLAB Network, they will be in position to play a key role to improve livestock health and productivity at the national and regional levels, namely in the West-Africa sub-region.

Field support mission to the Botswana National Veterinary Laboratory

The Botswana National Veterinary Laboratory (BVNL) is one of the leading laboratories of the VETLAB network. An APHL staff member travelled to Botswana to train 14 staff members on gene-based identification and characterization of animal pathogens, including multiplex assays for animal and zoonotic disease diagnosis, sequencing and bioinformatics. The training consisted of lectures and sixteen practical hands-on and problem-solving sessions covering molecular assay design and optimization, and analytical aspects for assay validation. It also addressed BNVL needs for the accreditation of molecular assays. Regarding sequencing, the participants were trained on sample preparation and the various steps of the sequencing process while working with sequencing service providers, stressing on quality checking, editing, and assembling of sequences. The participants also received training on how to perform sequence similarity searches, multiple sequence alignments, and phylogenetic reconstructions.

Technical support mission to the National Animal Health Diagnostics and Investigation Center, Ethiopia

The Luciferase Immunoprecipitation System (LIPS) for the highly sensitive and specific detection of antibodies against Peste des Petits Ruminants (PPR) in sheep and goat sera, an assay developed by APHL, was transferred to the National Animal Health Diagnostics and Investigation Center (NAHDIC) of Ethiopia. Personnel from NAHDIC and from the National Veterinary Institute (NVI) participated in the four-day long training during a field mission conducted from October 8th to 12th, 2018. Laboratory staff have now the capacity to operate the luminometer and use all reagents for testing sera using PPR LIPS. This gives NAHDIC a leading advantage in the region, as well as an additional means of fighting this disease, which is now the main target for a worldwide eradication campaign.

Field support mission to the Central Veterinary Laboratory, Kenya

As part of the Vet-lab Network project, an expert laboratory visit was conducted at the Central Veterinary Laboratories, Kabete, Kenya from October 15 to 19, 2018. The visit aimed at implementing molecular diagnostic protocols for the identification of transboundary animal diseases. During the five-day mission, the expert trained the personnel of CVL, Kabete on several protocols through demonstration and practical sessions. These included the conventional and Real-time RT-PCR for the identification of Newcastle disease; Real-time RT-PCR for African Swine Fever; Real-time RT-PCR for Peste des Petits Ruminants and a Multiplex Real-time RT-PCR for the identification of respiratory pathogens of small ruminants (PPRV, CCPP, P. multocida, Capripox). Reagents, controls and SOPs were also provided to CVL for the identification of Avipoxviruses, Infectious Bursal Diseases, Avian influenza and ruminant poxviruses.

Meetings

Technical meeting of veterinary laboratory directors of Asia and Africa participating in the VETLAB Network and **Third Research Coordination Meeting of CRP D32032 on Early Detection of Transboundary Animal Diseases to Facilitate Prevention and Control through a Veterinary Diagnostic Laboratory Network**

06 – 10 August 2018, IAEA Headquarters, Vienna, Austria.

The meeting brought together twenty-two Directors of VETLAB partners laboratories, as well as a representative of the World Organisation for Animal Health (OIE). The objectives of the meeting were to: (1) update the partners on the activities in 2017-2018; (2) discuss the 2018-2019 common and individual country plans; (3) address the production and sharing of reference material, validated procedures, interlaboratory testing and external quality assessment; (4) review and refine the VETLAB CRP objectives and the 2018-2019 work-plan; (5) facilitate exchange of experience, knowledge and information between the Asian and African Laboratories; and (6) formulate strategies for strengthening information sharing among VETLAB network partners through the VETLAB bulletin and the future VETLAB website. As in previous years, the VETLAB Directors met together with the VETLAB Research Coordination Meeting (RCM) participants. The meeting successfully reached its objectives and all VETLAB partners acknowledged the Joint FAO/IAEA programme for its significant contribution to these successes.

Training courses

Training Course on Diagnosis of Transboundary Animal Diseases: Sequencing and Bioinformatics Analysis of Animal Pathogen Genomes

10-21 September 2018, FAO/IAEA Laboratories, Seibersdorf, Austria.

Twenty-two participants from VETLAB partner laboratories in Africa and Asia attended this training course, designed to strengthen the capabilities of African and Asian Member States in genomic sequence data analysis for the diagnosis and identification of pathogens causing zoonotic and transboundary animal diseases. The analysis of sequence data can provide important information on the pathogens, including the potential source of introduction within a population in a specific geographical location. As sequencing cost drops, the technology becomes more accessible to MS laboratories which are increasingly using it to improve diagnostic efficiency. In this regard, the training targeted the improvement of the participants' skills in sequence analysis and bioinformatics and prepare them to better work with sequencing service providers. The training programme consisted of lectures and practical sessions on the applications bioinformatics to animal pathogens. The trainers were experts from the Istituto Zooprofilattico Sperimentale delle Venezie (Italy), Sciensano (Belgium) and the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

Regional Training Course on "Genetics of Parasite Resistance in Sheep and Goats: Application of Genomics and DNA Marker Information to Improve Small Ruminant Breeding"

24 September – 5 October 2018, FAO/IAEA Laboratories, Seibersdorf, Austria.

Gastro-intestinal parasitic infection is a major constraint for sheep rearing in Latin America, particularly with increasing concerns of anthelmintic resistance among parasites. The IAEA Technical Cooperation Department initiated a regional project in Latin America to implement breeding programs that focus on improving genetic potential of locally available sheep for enhanced parasite resistance characteristics. A hands-on practical training course was delivered to 20 participants from 12 countries in Latin America. It focused on genotyping workflow using real time PCR and microarray platforms, Genetics Laboratory Information and Data Management System, bioinformatics analysis of large sets of genomic data, implementing pedigree free animal models using molecular information and genome-wide association analysis related to parasite resistance in sheep. Each of the participant was provided with a package of multiple software tools for analysis of genetic data. It is expected that the

training will help the ongoing national efforts in the Latin American countries towards controlling the gastro-intestinal nematode parasites in sheep.

FIG. 10. Participants at the Regional Training Course on "Genetics of Parasite Resistance in Sheep and Goats: Application of Genomics and DNA Marker Information to Improve Small Ruminant Breeding".

Advanced "Train-the-Trainers" Course on the Diagnosis and Molecular Epidemiology of Transboundary Animal Diseases

05 - 16 November 2018, FAO/IAEA Laboratories, Seibersdorf, Austria.

Ten participants from VETLAB partner laboratories in Africa and Asia attended this training course. The objective of the course was to provide in-depth training to selected staff from laboratories partnering the VETLAB Network that are serving or will serve as trainers for other VETLAB Network members. The increasing need for training in various regions in Africa and Asia demands the establishment of a pool of trainers with up to date knowledge laboratory technologies. The advanced train-the-trainer course intended to prepare the potential trainers in better fulfilling their task of training other veterinary laboratory scientists.

During the first week, the participants received training on how to implement diagnostic strategies and troubleshoot molecular diagnostic test issues. In the second week, they studied the steps involved and the methods applied in phylogeny. Additionally, they learnt the Basics and practised scientific writing in English. The trainers were experts from Sciensano (Belgium), the University of Iowa, the Medical University of Vienna and the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

FIG. 11. Participants at the Advanced VETLAB Train-the-Trainers Course on the Diagnosis and Molecular Epidemiology of Transboundary Animal Diseases.

Fellowship and internship training

In 2018, the APHL hosted 12 interns/fellows in the following areas:

Name	Country	Status	Duration	Topic
ERECHU, Sam R. R.	Uganda	Fellow	2.5 months	Laboratory diagnosis and molecular epidemiology of transboundary animal diseases
HRISTOV, Kalin	Bulgaria	Fellow	1 week	Nuclear and nuclear related molecular technologies for early detection and confirmation of PPRV
KOYNARSKI, Tsvetoslav	Bulgaria	Fellow	2 months	Genetic evaluation of Bulgarian native cattle using DNA markers
KURUKULASURIYA, Maheshika	Sri Lanka	Fellow	2 weeks	Bioinformatics analysis of molecular genetic data for characterization of indigenous Sri Lanka sheep
MANOMOHAN, Vandana C. K.	India	Fellow	4 months	DNA marker based molecular characterization of South Indian zebu cattle
NIYONZIMA, Lineo R. B.	Lesotho	Fellow	1.5 months	Laboratory management and diagnosis of transboundary animal diseases
QUEMBO, Carlos J.	Mozambique	Scientific visit	1 week	Update on molecular epidemiology of transboundary animal diseases
SASSU, Elena L.	Italy	Intern	6 months	Immunology and immune-assays to evaluate immune response to vaccines in animals
STOIMENOV, Giorgi	Bulgaria	Fellow	1 week	Nuclear and nuclear related molecular technologies for early detection and confirmation of PPRV
TENEVA, Atanaska T.	Bulgaria	Fellow	1 week	Nuclear and nuclear related molecular technologies for genetic evaluation and characterization of livestock

Name	Country	Status	Duration	Topic
THEPPANGNANA, Whattana	Lao PDR	Fellow	1 months	Laboratory diagnosis of transboundary animal diseases using molecular and immunological techniques
ZAHARIEVA, Krasimera A.	Bulgaria	Scientific visit	1 week	Nuclear and nuclear related molecular technologies for genetic evaluation and characterization of livestock

PUBLICATIONS

SALEHI, B., MOTAMEDI-SEDEH, F., MADADGAR, O., KHALILI, I., GHALYAN, C.H.I., LANGROUDI, A., UNGER, H. AND WIJEWARDANA, V. (2018). Analysis of antigen conservation and inactivation of gamma-irradiated avian influenza virus subtype H9N2. Acta Microbiol Immunol Hung. 2018 Apr 24:1-9. doi: 10.1556/030.65.2018.025.

KANGETHE, R.T., PICHLER, R., CHUMA, F.N.J., CATTOLI, G. AND WIJEWARDANA, V. (2018). Bovine monocyte derived dendritic cell based assay for measuring vaccine immunogenicity in vitro. Vet Immunol Immunopathol. 2018 Mar; 197:39-48. doi: 10.1016/j.vetimm.2018.01.009.

PERELMAN, P.L., PICHLER, R., GAGGL, A., LARKIN, D.M., RAUDSEPP, T., ALSHANBARI, F., HOLL, H.M., BROOKS, S.A., BURGER, P.A. AND PERIASAMY, K. (2018). Construction of two whole genome radiation hybrid panels for dromedary (Camelus dromedarius): 5000RAD and 15000RAD. Scientific Reports 8, 1982 (2018) doi:10.1038/s41598-018-20223-5

MAPACO, L.P., LACERDA, Z., MONJANE, I.V.A., SUSSURO, A., VILJOEN, G.J., CATTOLI, G., DUNDON, W.G. AND ACHÁ, S.J. (2018). Molecular characterization of avipoxviruses circulating in Mozambique, 2016-2018. Arch Virol. 2018 May 8. doi: 10.1007/s00705-018-3864-0.[Epub ahead of print]

MOLINI, U., AIKUKUTU, G., KHAISEB, S., CATTOLI, G. AND DUNDON, W.G. (2018). Phylogenetic Analysis of Pigeon Paramyxoviruses Type-1 Identified in Mourning Collared Doves (Streptopelia decipiens) in Namibia, Africa. J Wildl Dis. 2018 Mar 29. doi:10.7589/2017-10-246.

DUNDON, W.G., ADOMBI, C.M., KANU, S., LOITSCH, A., CATTOLI, G. AND DIALLO, A. (2018). Complete Genome Sequence of a Lineage II Peste des Petits Ruminants Virus from Sierra Leone. Genome Announc. 2018 Jan 4;6(1). pii: e01417-17. doi: 10.1128/genomeA.01417-17.

CHIBSSA, T.R., GRABHERR, R., LOITSCH, A., SETTYPALLI, T.B.K., TUPPURAINEN, E., NWANKPA, N., TOUNKARA, K., MADANI, H., OMANI, A., DIOP, M., CATTOLI, G., DIALLO, A. AND LAMIEN CE. (2018). A gel-based PCR method to differentiate sheeppox virus field isolates from vaccine strains. Virol J. 2018 Apr 2;15(1):59. doi: 10.1186/s12985-018-0969-8.

CHAUDHRY, M., RASHID, H.B., ANGOT, A., THRUSFIELD, M., BRONSVOORT, B.M.D., CAPUA, I., CATTOLI, G., WELBURN, S.C. AND EISLER, M.C. (2018). Risk Factors for Avian Influenza H9 Infection of Chickens in Live Bird Retail Stalls of Lahore District, Pakistan 2009-2010. Sci Rep. 2018 Apr 4;8(1):5634. doi: 10.1038/s41598-018-23895-1.

LUKA, P.D., SHAMAKI, D., LAMIEN, C.E., NYAM, D.C., ACHENBACH, J.E., JAMBO, A.R., YAKUBU, B. AND UNGER H. (2018). Experimental infection and direct blood detection of African swine fever in pigs. 4th Annual GARA Scientific Workshop, Cagliari-Sardinia, Italy, April 11-13, 2018
LUKA, P.D., SHAMAKI, D., MWIINE, F.N., LAMIEN, C.E., ACHENBACH, J.E., ERUME, J., YAKUBU, B. AND UNGER, H. (2018). Spatio-temporal emergence of several tetrameric repeats of African swine fever

genotype I from Nigeria. 4th Annual GARA Scientific Workshop, Cagliari-Sardinia, Italy, April 11-13, 2018

WIJEWARDANA, V., KANGETHE, R., SASSU, E.L., PICHLER, R. AND CATTOLI, G. (2018). Dendritic cell based in-vitro assay to test immunogenicity of viral vaccine candidates. Proceedings of the 11th International Congress for Veterinary Virology, Vienna, 27-30 August 2018.

DUNDON, W.G., DONDUASHVILI, M., GIOGINASHVILI, K., TOKLIKISHVILI, N., TIGILAURI, T., GELASHVILI, L., AVALIANI, L., KHARTSKHIA, N., LOITSCH, A., BATAILLE, A., LIBEAU, G., DIALLO, A. AND CATTOLI, G. (2018). Identification of Peste des Petites Ruminants, Georgia. Proceedings of the 11th International Congress for Veterinary Virology, Vienna, 27-30 August 2018.

BERGUIDO, F.J., GELAYE, E., KRSTEVSKI, K., LOITSCH, A., COMTET, L., POURQIER, P., TUPPURAINEN, E., DIALLO, A., CATTOLI, G., LAMIEN, C.E. (2018). Evaluation of capripox surface protein as antigens for the detection of antibodies using indirect ELISA. Proceedings of the 11th International Congress for Veterinary Virology, Vienna, 27-30 August 2018.

SETTYPALLY, T.B.K., TOUNKARA, K., LOITSCH, A., DIALLO, A., CATTOLI, G. AND LAMIEN, C.E. (2018). Syndromic surveillance and pathogens detection using multiplex assays for respiratory infections in small ruminants. Proceedings of the 11th International Congress for Veterinary Virology, Vienna, 27-30 August 2018.

CHIBSSA, T.R., SETTYPALLY, T.B.K., BERGUIDO, F.J., LOITSCH, A., DIALLO, A., CATTOLI, G. AND LAMIEN, C.E. (2018). Differentiation of sheep pox virus vaccines from field isolates and other capripox species. Proceedings of the 11th International Congress for Veterinary Virology, Vienna, 27-30 August 2018.

CATTOLI, G. AND LAMIEN, C.E. (2018). Lumpy Skin Disease: an emerging animal disease in Europe. Proceedings of the European College of Bovine Health Management / 50th Congress Italian Association of Bovine Medicine, Italy, 11-13 October 2018.

CHIBSSA, T.R., GRABHERR, R., LOITSCH, A., SETTYPALLI, T.B.K., TUPPURAINEN, E., NWANKPA, N., TOUNKARA, K., MADANI, H., OMANI, A., DIOP, M., CATTOLI, G., DIALLO, A. AND LAMIEN, C.E. (2018). Agel-based PCR method to differentiate sheeppox virus field isolates from vaccine strains. Virol J. 2018 Apr 2;15(1):59. doi: 10.1186/s12985-018-0969-8.

MOLINI, U., AIKUKUTU, G., KHAISEB, S., CATTOLI, G. AND DUNDON, W.G. (2018). Phylogenetic Analysis of Pigeon Paramyxoviruses Type-1 Identified in Mourning Collared Doves (Streptopelia decipiens) in Namibia, Africa. J Wildl Dis. 2018 Mar 29. doi:10.7589/2017-10-246.

MOLINI, U., AIKUKUTU, G., KHAISEB, S., HAINDONGO, N.N., LILUNGWE, A.C., CATTOLI, G., DUNDON, W.G. AND LAMIEN, C.E. (2018). Molecular characterization of lumpy skin disease virus in Namibia, 2017. Arch Virol. 2018 Jun 4. doi: 10.1007/s00705-018-3891-x.

WADE, A., JUMBO, S.D., ZECCHIN, B., FUSARO, A., TAIGA, T., BIANCO, A., RODRIGUE, P.N., SALOMONI, A., KAMENI, J.M.F., ZAMPERIN, G., NENKAM, R., FOUPOUAPOUOGNIGNI, Y., ABDOULKADIRI, S., ABOUBAKAR, Y., WIERSMA, L., CATTOLI, G. AND MONNE, I. (2018). Highly Pathogenic Avian Influenza A(H5N8) Virus, Cameroon, 2017. Emerg Infect Dis. 2018 Jul;24(7):1367-1370. doi: 10.3201/eid2407.172120.

WADE, A., TAÏGA FOUDA, M.A., MAIMOUSSA, A., JEAN MARC, F.K., NJOUOM, R., VERNET, M.A., DJONWE, G., MBALLA, E., KAZI, J.P., NENKAM, R., POUEME NAMEGNI, R., BAMANGA, H., CASIMIR, N.K.M., LEBRETON, M., NWOBEGAHAY, J., FUSARO, A., ZECCHIN, B., MILANI, A., GASTON, M., CHEPNDA, V.R., DICKMU JUMBO, S., SOULEY, A., ABOUBAKAR, Y., FOTSO KAMNGA, Z., COUO, K., ATKAM, H., DAUPHIN, G., WIERSMA, L., BEBAY, C., NZIETCHUENG, S., VINCENT, T., BIAOU, C., MBACHAM, W., MONNE, I. AND CATTOLI G. (2018). Highly pathogenic avian influenza A/H5N1 Clade 2.3.2.1c virus in poultry in Cameroon, 2016-2017. Avian Pathol. 2018 Jul 9:1-51. doi:10.1080/03079457.2018.1492087.

BONFANTE, F., MAZZETTO, E., ZANARDELLO, C., FORTIN, A., GOBBO, F., MANIERO, S., BIGOLARO, M., DAVIDSON, I., HADDAS, R., CATTOLI, G. AND TERREGINO C. (2018). A G1-lineage H9N2 virus with oviduct tropism causes chronic pathological changes in the infundibulum and a long-lasting drop in egg production. Vet Res. 2018 Aug 29;49(1):83.

CAPUA, I., MERCALLI, A., ROMERO-TEJEDA, A., PIZZUTO, M.S., KASLOFF, S., SORDI, V., MARZINOTTO, I., LAMPASONA, V., VICENZI, E., DE BATTISTI, C., BONFANTI, R., RIGAMONTI, A., TERREGINO, C., DOGLIONI, C., CATTOLI G. AND PIEMONTI, L. (2018). Study of 2009 H1N1 Pandemic Influenza Virus as a Possible Causative Agent of Diabetes. J Clin Endocrinol Metab. 2018 Dec 1;103(12):4343-4356.

TOUNKARA, K., BATAILLE, A., ADOMBI, C.M., MAIKANO, I., DJIBO, G., SETTYPALLI, T.B.K., LOITSCH, A., DIALLO, A. AND LIBEAU, G. (2018). First genetic characterization of Peste des Petits Ruminants from Niger: On the advancing front of the Asian virus lineage. Transbound Emerg Dis. 2018 Oct;65(5):1145-1151. doi: 10.1111/tbed.12901.

TSHILENGE, G.M., DUNDON, W.G., DE NARDI, M., MULUMBA MFUMU, L.K., RWEYEMAMU, M., KAYEMBE-NTUMBA, J.M. AND MASUMU, J. (2018). Seroprevalence of Rift Valley fever virus in cattle in the Democratic Republic of the Congo. Trop Anim Health Prod. 2018 Oct 22. doi:10.1007/s11250-018-1721-5.

DONDUASHVILI, M., GOGINASHVILI, K., TOKLIKISHVILI, N., TIGILAURI, T., GELASHVILI, L., AVALIANI, L., KHARTSKHIA, N., LOITSCH, A., BATAILLE, A., LIBEAU, G., DIALLO, A. AND DUNDON, W.G. (2018). Identification of Peste des Petits Ruminants Virus, Georgia, 2016. Emerg Infect Dis. 2018 Aug;24(8):1576-1578. doi: 10.3201/eid2408.170334.

TENEVA, A., TODOROVSKA, E., PETROVIC, M.P., KUSZA, S., PERIASAMY, K., PETROVIC, V.C., ANDRIC, D.O. AND GADJEV, D. (2018). Short tandem repeats (STR) in cattle genomics and breeding. Biotechnology in Animal Husbandry, 34: 127-147

Book Chapter.

BABIUK, S., DIALLO, A., LAMIEN, C.E. AND KITCHING, R.P. (2018). Sheeppox and goatpox. In: JAW Coetzer, GR Thomson, NJ Maclachlan, M-L Penrith and A Michel (Eds), Infectious Diseases of Livestock. Anipedia, www.anipedia.org.

VETLAB NETWORK

The APHL provides important contributions to the VETLAB Network, a network of national veterinary diagnostic laboratories from 45 African and 19 Asia and Pacific Member States. The VETLAB network is coordinated by the APH Subprogramme and supported through FAO/IAEA programmatic activities as well as by South Africa through the African Renaissance Fund and USA and Japan through the IAEA's Peaceful Uses Initiative. APHL contributed to several VETLAB Network events aiming at building diagnostic capacities and sharing information and expertise. Among the major events were: the Annual VETLAB Laboratory Directors Meeting (Vienna, Austria, August 2018), the training course on Diagnosis of Transboundary Animal Diseases: Sequencing and Bioinformatics Analysis of Animal Pathogen Genomes (Seibersdorf, Austria, September 2018), and the Advanced Train-the-Trainers Course on the Diagnosis and Molecular Epidemiology of Transboundary Animal Diseases (Seibersdorf, Austria, November 2018). Five VETLAB partners laboratories were visited and their staff trained during ad-hoc field missions (Bangladesh, Botswana, Ethiopia, Kenya, Zambia). The APHL also provides continuous on-site and on-line support to partner laboratories on disease diagnosis, outbreak investigations, laboratory troubleshooting, and contributes to the VETLAB Network Bulletin, a forum for participating laboratories and other stakeholders to communicate and exchange knowledge/information, to showcase achievements and to share expertise within the VETLAB Network.

EXTRA-BUDGETARY SUPPORT

AFRICAN RENNAISANCE FUND: 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.

IAEA's PEACEFUL USES INITIATIVE: The improvement of and capacity building in nuclear and nuclear related animal disease diagnostic capacities of veterinary laboratories in Africa and Asia; funded by the United States' Department of State and by the Government of 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 and authenticity determination and for contaminant control systems. 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 and the development, validation, transfer and application of nuclear and related methods for testing foods. The application of these technologies and methods in Member States is supported by the development and provision of technical protocols, advice and guidance, training both in the FEPL and in Member States, and providing input for the development of international standards.

Research and development achievements in 2018 included the development and evaluation of analytical methods for food authentication and to underpin food traceability systems, and to control residues and contaminants in food. The focus was on important commodities in international trade and targets for fraudulent practices such as counterfeiting or adulteration. Method development encompassed both rapid screening methods and more sophisticated techniques, in order to provide Member States with the options needed for their particular food control systems.

A study was carried out into the potential use of carbon, nitrogen and sulphur stable isotope analysis for the verification of Malysian edible bird's nest. Rapid screening methods utilizing hand-held or portable instruments (Fourier-transform infrared spectroscopy (FTIR), Raman spectroscopy and near-infrared spectroscopy (NIR)) were developed for the detection of formaldehyde in milk, differentiation of geographic origin and type of teas, and detection of adulteration of argan oil. All these methods included advanced chemometric techniques for data analysis and interpretation. Methodology for food contaminant control included the development of a multi-residue/contaminant method to detect pesticides, veterinary drugs and other chemicals in honey, with assistance of two fellows from Palestine, which was applied to honey samples from Palestine. A study was completed for the verification of the homogeneity of the analytical portion following the sample processing step of a method for pesticide residues in vine leaves, as part of a method validation study in collaboration with scientists from Syria and Uruguay. The results of the R&D programme are made available through scientific publications, online method protocols and via laboratory networks such as the Red Analitica de Latinoamérica y el Caribe (RALACA) and those involved in technical cooperation and research projects.

Two EU projects in which FEPL participated were completed in 2018. Outputs from the EU 7th Framework integrated project 'FoodIntegrity' included a web resource, the 'Food Integrity Knowledge Base' of food authenticity issues and methodologies, and a series of scientific opinions on a range of topics related to food authenticity and fraud detection. Outputs from the EU Horizon 2020 project 'Authent-Net' included the web-based Food Authenticity Research Network Hub (FARNHub,) which provides registered users with an overview of currently available resources related to food authenticity, and a low-level European voluntary standard, CEN (Comité Européen de Normalisation) Workshop Agreement CWA 17369:2019, 'Authenticity and fraud in the feed and food chain - Concepts, terms, and definitions', which was. published on 23 January 2019.

The FEPL coordinated and provided technical input to two coordinated research projects (CRPs) on food traceability and authenticity, involving approximately thirty countries. One of the projects was successfully completed in 2018, with over 160 individual outputs produced by 10 contract holders.

The results of FEPL research were presented at five international conferences, and the FEPL was represented in the scientific committees for five major international conferences on food safety. The laboratory took part in the Austrian 'Long night of research'. The FEPL also contributed as a member of the UK's Food Authenticity Methodology Working Group and the advisory board of the ASSET (Assured, Safe and Traceable Food) Centre at Queen's University Belfast, UK.

The FEPL provided technical input to twenty-two national and five regional TCPs in 2018. Human resource capability was enhanced through the training of 418 scientists, analytical chemists, laboratory personnel and food inspectors in 16 courses, workshops or seminars. The FEPL hosted two interns, three fellows, and two scientific visitors during 2018. Technical backstopping, advice and contributions to webinars and other activities were provided to the RALACA network of food safety laboratories in Latin America and the Caribbean.

Publications by FEPL staff in 2018 comprised two books, including a laboratory manual comprising thirty analytical methods, five book chapters, four papers in peer-reviewed scientific journals, and eight conference papers, abstracts or reports.

STAFF

Name	Title
Cannavan, Andrew	Laboratory Head
Kelly, Simon	Food Safety Specialist
Maestroni, Britt Marianna	Food Scientist
Jandrić, Zora	Analytical Chemist
Mihailova, Alina	Analytical Chemist
Islam, Marivil	Laboratory Technician
Abrahim, Aiman	Laboratory Technician
Beckham, Stephanie	Team Assistant
Osmic, Melika	Team Assistant
Vaughan, Amber	Intern
Xu, Xiao	Intern
Jin, Shunru	Cost-free Fellow

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

The research and development activities in the Food and Environmental Protection Laboratory (FEPL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture focus on the development or adaptation of analytical methods to help Member States to improve their food control systems. The issues of importance to Member State governments, regulators, industry and ultimately consumers can be summarized in the three questions, "is my food safe?", "am I eating what I think I'm eating?", and "am I getting what I paid for?". The analytical methods developed in the FEPL and transferred to Member State laboratories, therefore, include methods for the detection and quantification of chemical residues and contaminants in food (e.g. pesticides, veterinary drug residues), for testing various criteria related to the authenticity of foods, such as confirmation of stated geographical origin, production technique (e.g. organically produced foods), quality (e.g. extra virgin olive oil as opposed to processed olive oil) and adulteration (e.g. dilution of extra virgin olive oil with vegetable or nut oils, dilution of honey with corn syrup). Method development in the FEPL encompasses both rapid screening methods and more sophisticated techniques, in order to provide Member States with the options needed for their particular food control systems FEPL 2018 contributions in terms of R&D are presented below under two main categories: (i) food traceability and authenticity and (ii) control of residues and contaminants in food, along with key highlights from coordinated research projects.

Food traceability and authenticity

A study into the potential use of carbon, nitrogen and sulphur stable isotope analysis of Malaysian edible bird's nests (EBNs) to verify their provenance

For most food products the authentic item is distinguished by botanical, cultivar, geographical or production origin and/or the absence of adulterants. In the case of edible bird's nest (EBN), the economic motivation for adulteration and mislabelling of origin is significant as EBNs rank amongst the world's most expensive animal products for food and traditional medicinal uses. For example, in Thailand the price of white EBN reached 65,000 Baht (US$ 2,170) per kilogram in 2007 and had a total export value of approximately 126 million Baht (US$ 4.2 million) per annum. Authentication and assessment of EBN quality may be completed by various targeted chemical methods, which confirm that the product quality meets technical and compositional regulatory requirements. One important aspect of EBN quality that has not been extensively reported relates to its geographical origin. We previously described an untargeted method for the classification of EBN by geographical origin using Fourier transform infrared - attenuated total reflectance spectroscopy which showed potential as a rapid screening technique for use in the field. However, additional analytical methodology is required to complement and verify such screening techniques and provide some linkage to the environmental factors that allow discrimination of the EBN by region, such as possible variations in the chemical composition of EBNs due to different breeding sites, climate and swiftlets' (Aerodramus fuciphagus and Aerodramus maximus) dietary intake.

When seeking to verify labelling claims of geographical origin of food, bulk stable isotope analysis of hydrogen, carbon, nitrogen and sulphur has been identified as one of the most promising analytical tools to link a food's isotopic characteristics to the biogeochemical signature of its cultivation or production zone. Broadly speaking the position of the swiftlet in its local food web will affect nitrogen and carbon stable isotope ratios in its tissues and the saliva that eventually makes up EBNs. Consequently, we may reasonably expect the nitrogen stable isotope values to reflect the insectivorous nature of the swiftlets' feeding. Furthermore, the relative proportions of C3 and C4 dietary inputs in the insects consumed by the swiftlets will be reflected in the birds' tissues, including the trophic shift in carbon isotope values associated with their position in the food chain. Ultimately, this will present a pattern of fractionation that could potentially be usefully exploited for geographical origin assignment.

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Carbon and nitrogen stable isotope data (δ^{13}C and δ^{15}N) from authentic Malaysian EBNs

Bulk carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope measurements of EBN were made using an elemental analyser coupled to a stable isotope mass spectrometer. The EBN measured δ^{13}C values covered a range of -26.99‰ to -23.80‰ and the δ^{15}N values covered a range of 5.05‰ to 8.06‰. The carbon isotope values are typical of Calvin (C_3) plant sources. The swiftlets, from which the nests are commonly gathered, are insectivorous. The nitrogen isotope values cover 1 trophic level (~3‰) and therefore it appears likely that the swiftlets feed on relatively small primary consumer insects and not larger predatory insects that would have given rise to a wider and more positive range of nitrogen isotope values. This is also evidenced by the substantial overlap of δ^{15}N values from the different locations.
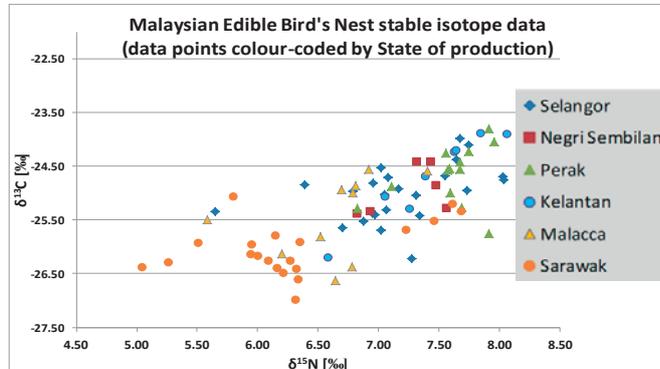
FIG. 1: *A graph showing the δ^{13}C and δ^{15}N values obtained from 81 authentic EBNs produced in different States of Malaysia*

Visual inspection of the data shown in Fig. 1 suggests a correlation between δ^{13}C and δ^{15}N values for the EBNs; a t-test confirmed statistically significant correlation (α =0.01).

Prediction ellipses (95%) were calculated and plotted to illustrate the limited separation between EBN production locations based solely on δ^{13}C and δ^{15}N values. Examples of two of the plots (Perak/Sarawak and Selangor/Sarawak) are shown in Fig.2; separation between other locations was similar.
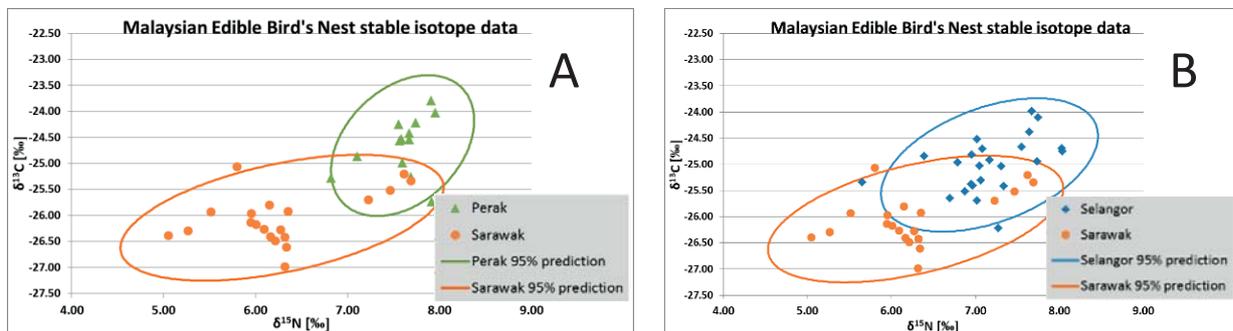
FIG. 2: *δ^{13}C and δ^{15}N values for the EBNs produced in the Malaysian States of (A) Perak and Sarawak and (B) Selangor and Sarawak*

Addition of sulphur isotope data (δ^{34}S)

It is generally accepted that animals are not capable of reducing sulphate and synthesizing complex sulphur containing bio-molecules. Consequently, they receive most of their sulphur compounds via their diet, and the amino acids methionine and cysteine, to some extent, are essential for most animals. Furthermore, sulphate reduction does not produce significant fractionation, so organic sulphur is clearly related to its source. Therefore, the soil or sulphate fertiliser from which it is derived can provide useful geographical origin information.

Most of the sulphur containing organic molecules, such as the sulphur amino-acids, in plants and animals occur at very low concentrations. This means that reliably measuring sulphur isotope ratios in plant and animal products has presented a significant technological challenge that was previously overcome by time-consuming enrichment of the sulphur concentration through isolation of specific amino acids after hydrolysis and separation by preparative chromatography. More recently, it has been possible to measure sulphur isotope data (δ^{34}S) without enrichment using an elemental analyser equipped with a sulphur dioxide trap and purge system coupled to a stable isotope mass

spectrometer. Using this EA-IRMS system in FEPL, the δ^{34}S values measured in the authentic Malaysian EBN samples were found to cover a wide range of values from +1.9‰ to +14.3‰ with a mean value of +7.8‰. Generally higher δ^{34}S values were observed for EBN produced in Sarawak compared to the EBN produced in the other states of Kelantan, Malacca, Negri Sambilan, Perak and Selangor. This may be explained by the geographical separation of the South China Sea between the State of Sarawak and the remaining production states.

Stable carbon, nitrogen and sulphur (CNS) isotope analysis of Malaysian EBNs has shown potential for distinguishing between the various regions in which they are produced, based on preliminary data. Combining the CNS isotope data from the authentic Malaysian EBN and using a canonical discriminant model permits a correct classification rate of around 50%. Using additional stable isotope data such as that derived from hydrogen and/or oxygen and combining trace element profiling would likely improve the reliability of the determination of geographical origin and classification rates. Consequently, further research is required to establish the usefulness of δ^{2}H and/or δ^{18}O stable isotope analysis of EBN to discriminate between the Malaysian States of production and perhaps more importantly differentiate them from EBN produced in other countries such as Indonesia, Philippines and Thailand. The temporal stability of these models and the effects of different technological also need to be established.

Screening methods to detect formaldehyde adulteration of liquid milk

Milk is a primary source of nutrients for many consumers around the world and with a growing population, there is an increasing demand for its production. To satisfy this demand and increase profits, milk may be artificially extended through dilution or adulterated to increase nitrogen (apparent protein) or fat content and prolong shelf life. One recent example is the adulteration of milk in Brazil, where commercially available UHT milk was reported to contain formaldehyde (a known carcinogen), hydrogen peroxide and chlorine. Preservatives such as formaldehyde, hydrogen peroxide, hypochlorite, etc. are often used to mask poor hygienic quality in milk production or to prevent rancidity when there is a lack of chilled transport or storage. As guidelines are established that define the maximum content of certain adulterants, there is a continued demand to develop rapid detection techniques that can keep up with constantly evolving and more sophisticated milk adulteration methods.

Authentication and assessment of milk quality may be completed by various targeted chemical methods, which confirm that the product quality meets technical and regulatory requirements. Typically, this analysis demands relatively labour-intensive sample preparation and time consuming sequential analytical measurements. An alternative approach is to conduct an untargeted quick, relatively cheap, and often non-destructive spectroscopic measurement with subsequent data processing by means of chemometrics.

Portable Raman spectroscopy

Raman spectrometry is an example of a rapid and portable screening method that, when combined with chemometrics, can potentially identify and extract the features of interest from the acquired spectrum to allow differentiation between adulterated and authentic product. The Raman measurements are rapid, simple and need no sample preparation. Raman spectroscopy measures vibrational, rotational, and other low-frequency modes in molecules and is commonly used to create a structural 'fingerprint' of the chemicals present. This fingerprint can be used to identify and quantify chemicals present in a sample. The Raman spectrum region 800-900nm, especially for milk, is able to identify important components and the absorption bands are sensitive to the physical and chemical states of individual constituents.

Within the framework of the coordinated research project "Field-deployable analytical methods to assess the authenticity, safety and quality of food" (D52040/G42007), a study into the use of a portable Raman spectrometer was undertaken in FEPL to demonstrate the feasibility of field-based testing of liquid milk to detect the presence of the toxic and carcinogenic preservative formaldehyde. Raman spectra were obtained using the Portable StellarCASE-Raman Instrument and SpectraWiz software. Data was assessed using data-driven soft independent modelling by one-class analogy (DD-SIMCA) with 'chemometrics add-in for Excel' software. Formaldehyde solution was added to whole liquid ultra-heat treated (UHT) milk to create artificial fortification levels between 0.2 and 10% (v/v)
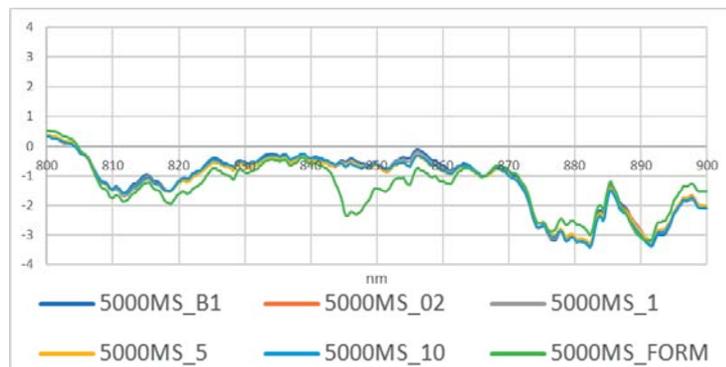

FIG 3: *Single Normal Variate transformed Raman spectra over distinct fingerprint region (800-900nm) averaged from 2 days for each spiking level*

formaldehyde. Spectra (794-1200 nm) were collected in low resolution % transmittance mode. Each Raman spectrum was normalised by single normal variate (SNV) pre-processing before modelling using DD-SIMCA. Artificially adulterated milks and pure adulterant were compared to the model separately and the suitability of the model was assessed against a target sensitivity and specificity rate of 95%, both with a significance level of p=0.01. Typical averaged Raman spectra are shown in Fig. 3.

Results from DD-SIMCA modeling showed that the detection of formaldehyde was possible at 0.2% (v/v) adulteration. This is well below concentrations of formaldehyde that are typically used to preserve milk. The previously mentioned Brazilian UHT milk example contained 44% formaldehyde.

Very near infrared 'pocket molecular sensor'

Recent advances in the miniaturisation of scientific equipment have permitted the production of small sensors capable of measuring the spectrum produced from various food commodities. One such device is a hand-held near-infrared (NIR) spectrometer, the SCIO 'pocket molecular sensor', which is capable of recording a spectrum in the very near infrared (750 and 1050 nm) and then comparing it against a database of spectra from authentic food products through a mobile phone or tablet linked to the internet. The feasibility of using this device to rapidly test liquid milk for the presence of formaldehyde was investigated.

Fresh full-fat liquid milk was fortified with formaldehyde solution at concentrations of 0.2%, 1%, 5% and 10% (v/v) and analysed using the SCIO. A model was created by combining data from the unfortified (blank) and fortified samples and the formaldehyde solution.

A classification model was created after pre-processing of the spectra, taking the log of the spectral data, subtracting the average and then taking the second derivative. This provided the best separation between the whole milk and milk fortified with formaldehyde at the 5% and 10% (v/v) concentration, but not for samples fortified at 1% (v/v) or less (Fig. 4). When tested at the lower concentrations, the model misclassified some of the test materials; with some blanks being identified as containing low levels of formaldehyde and some 0.2% (v/v) samples identified as not containing formaldehyde.

Due to the limited number of samples studied here the results from the Raman and SCIO studies can be considered only as preliminary. Further research is required to significantly increase the number of


	Samples	Scans
0% FAH in Milk	30	90
0.2% FAH in Milk	30	88
1% FAH in Milk	30	90
5% FAH in Milk	30	90
10% FAH in Milk	30	91
36.50% FAH	18	54

(FAH = formaldehyde)
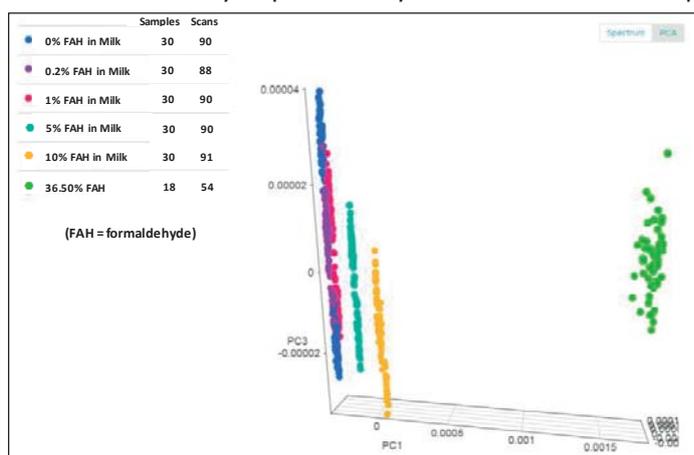
FIG. 4: *Classification model constructed from the spectra obtained using the SCIO 'pocket molecular sensor'*

authentic milk samples to ensure that the databases used for comparison of market and imported samples reflect the natural variation in milk chemical composition that occurs due to seasonal changes in cattle feed and technological processing. Nevertheless, the feasibility of using both Raman spectroscopy and the SCIO 'pocket molecular sensor', combined with chemometrics, for the detection of low concentrations of the toxic and carcinogenic preservative formaldehyde in whole liquid milk has been successfully demonstrated. These techniques are accessible, non-destructive, fast and direct, requiring no sample preparation.

Additional work is currently underway to detect adulteration of liquid milk with formaldehyde and a range of other adulterants including, boric acid, detergent, melamine, urea, sugar, salt and others.

Differentiation of geographic origin and type of tea by hand-held infrared spectroscopy

Tea is one of the most popular and widely consumed beverages in the world. There are many different tea categories, including green tea, black tea, oolong tea, yellow tea, white tea and dark compressed tea. Green and black teas are among the most popular categories. These varieties can differ substantially due to factors such as variable growing conditions, horticulture, processing and harvesting time. There are large variations in both price and quality, leading to misrepresentation on the market.

Various techniques have been applied for the quality control of tea, based on the identification and quantification of organic constituents and mineral content, but the chemical analysis methods used can be very expensive and time-consuming. Rapid, accurate and convenient analytical methods are required to differentiate geographical origins for the quality control of tea at production or market level.

A hand-held SCIO 'pocket molecular sensor' was tested for differentiation of tea varieties and the geographical origin of different teas. Without any sample preparation, samples of authentic black tea and green tea were scanned using the handheld device. The raw data were processed using the Scio-lab app to build predictive models for classification. Principal components analysis was used to check for groupings/clustering using several pre-processing methods. After selecting the best combination (SNV and 1st derivative), a random forest algorithm was applied to create the classification model.

The PCA plot (Fig. 5) showed good separation of black tea by country and even by product brand within the same country. A classification model was created which gave an F1 score of 0.9999

|	Samples	Scans
Ceylon	12	36
Ceylon 2	12	36
China	12	36
China Jiangsu	12	36
India	12	36
India 2	12	36
Nepal	12	36
South Africa	12	36
Sri Lanka	12	36
Taiwan	12	36
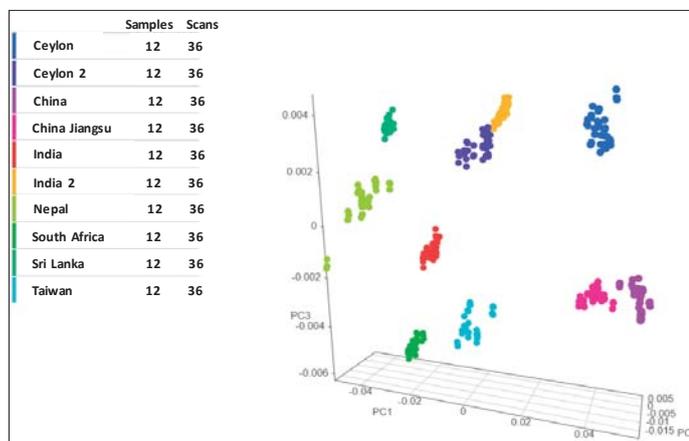
FIG. 5: *3-D PCA plot of black tea by country of origin*

indicating a very good model accuracy. The performance of the chemometric models created was evaluated by running test samples on a different day. The model constructed for geographical origin of black tea correctly predicted the origin of about 90% of the samples tested.

Although the number of samples was very limited, the results suggest that the SCIO handheld NIR spectrometer could potentially be used as a rapid technique to provide information on the authenticity, with respect to geographical origin, of teas.

Detection of adulteration of argan oil by Fourier-transform infrared spectroscopy

The argan tree (Argania spinosa (L.) Skeels; Sapotaceae) is a slow-growing tree native to Morocco. In recent years, argan oil has become one of the most expensive oils in the world due to its delicate hazelnut taste and multiple pharmacological properties. It is used in the food industry and also incorporated into many cosmetic products. Argan oil is now recognised as a Protected Geographical Indication product of Morocco and because of its high value, is a target for economically motivated adulteration. One of the most common adulterations encountered in argan oil is the addition of sunflower oil, because of its relative cheapness. Therefore, the need has arisen for effective and rapid quality control methods aiming at detecting the adulteration of argan oil.

Fourier-transform infrared spectroscopy (FTIR) is a reliable and well recognized fingerprinting method for providing information about molecular structure and composition. Attenuated total internal reflectance (ATR) is an accessory used with the FTIR spectrophotometer to measure surface properties of solid or thin film samples rather than their bulk properties. The FTIR–ATR technique has been proven as an effective tool in a wide range of food adulteration problems.

A study was initiated to investigate the potential of FTIR-ATR for the detection of adulteration of argan oil with sunflower oil. Assumed authentic argan oil samples were provided by a project counterpart in Morocco, along with argan oils adulterated with sunflower oil at 20%, 30% and 50% (v/v) in the laboratory in Morocco. Spectra were acquired in the wavenumber range 4000-450 cm^{-1} and processed through Perkin Elmer Spectrum software. Processed data were further analysed using Perkin Elmer Spectrum Quant software and chemometrics in Excel.

Using the experimental results, a one-class DD-SIMCA classification model for argan oil showed a detection limit of 20% adulteration of argan oil with sunflower oil. This preliminary study demonstrated the feasibility of using FTIR-ATR to rapidly screen argan oil for the presence of adulterants such as sunflower oil. Further research is required to increase the size of the authentic argan oil database to incorporate factors such as inter-annual variation in production, possible technological processing effects and the effects of longer-term storage.

Control of residues and contaminants in food

A multi contaminant method for honey: from method development to validation

It is well known that bees are indicators of environmental contamination since they can carry into the hive different pollutants and agrochemicals they encounter in the fields. In addition, antimicrobials are still frequently directly applied in the hive to control honey bee diseases. The issue of authorization of antimicrobials for use in apiculture is not harmonised among FAO or IAEA Member States. There is, therefore, a need for a multi-contaminant and multi-class analytical method to ensure safe honey for consumers.

The development and validation of a multi-contaminant and multi-class analytical method for honey samples was initiated in FEPL in August 2008, with two scientists from Palestine on IAEA technical cooperation fellowships. The study complements previous work in FEPL on honey authenticity by enabling testing for the presence of contaminants in floral honeys and therefore contributing to the safety of honey on the market.

Honey can be contaminated with various types of substances, including pesticides, veterinary drugs, heavy metals and even mycotoxins. Since many different classes, each comprising many analytes with differing physico-chemical properties, exist within each of these types of contaminants, analysis becomes a real challenge. For this study, thirty-five target analytes were selected for initial method development, including pesticides, veterinary drugs and one marker of authenticity that was identified in previous work. A generic and versatile sample preparation technique, the QuEChERS method, was employed. Method development required an understanding of the interactions between the analytes and the honey matrix. Honey is a very complex matrix containing large amounts of sugars, enzymes and proteins. The goal of the sample preparation step is to decrease the number of co-extractives while concentrating the analytes of interest. In this method, despite all efforts to reduce matrix co-extractives, the matrix effects were still very high in the acetonitrile extracts, as can be seen in Fig. 6 for two analytes with very different properties, furazolidone (a nitrofuran veterinary drug) and carbendazim (a benzimidazole pesticide). The matrix effects for both compounds were estimated to be of the order of 420%. This indicates that careful calibration is needed to quantify the target analytes, typically using matrix-matched calibration or isotopically-labeled internal standards. While method optimization is ongoing preliminary data indicate that the method can be validated at 0.01 mg/kg employing a sensitive liquid chromatography - tandem mass spectrometry instrument. Although not yet fully validated, the method was successfully applied to test the contamination levels of 3 honey samples originating from Palestine.
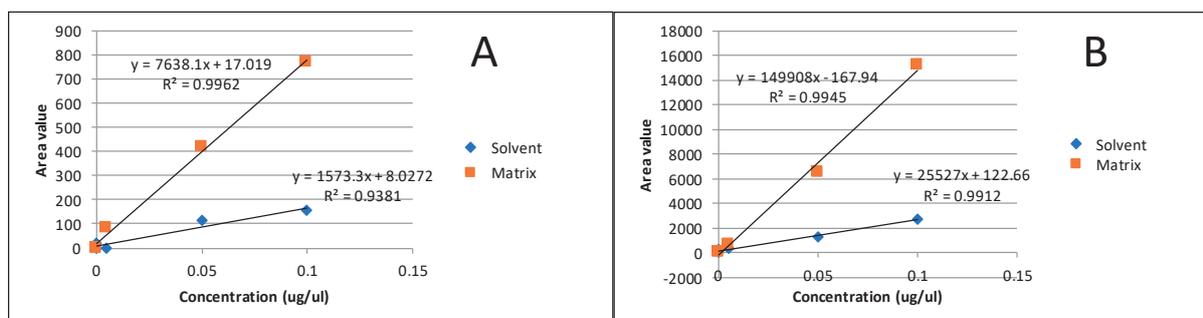

FIG. 6: The difference in the slope for the regression lines for the solvent calibration and the matrix matched calibration demonstrates the matrix effects for (A) furazolidone and (B) carbendazim in honey

Homogeneity of sample processing

The FEPL frequently receives requests from Member State laboratories for assistance on different aspects of method validation. Validation is the process of characterising the performance of a method to demonstrate that it is fit for purpose. One aspect of validation that is often neglected is sample processing. Sample processing should ensure homogeneity of the analytes in the processed commodity, so that the analytical result from a defined analytical portion is as representative as possible of the level of analyte in the bulk sample. Sample processing is defined as the procedure (e.g. cutting, grinding, mixing) used to make the analytical sample acceptably homogeneous with respect to the analyte distribution, prior to removal of the analytical portion. The FEPL, as part of its method validation studies for pesticides residues in vine leaves in collaboration with the Syrian Atomic Energy Commission, and the Grupo de Análisis de Compuestos Traza (GACT), Uruguay, initiated a study on the verification of the homogeneity of the 2 g analytical portions specified in the method. Two different approaches were adopted to verify that the sample processing procedure produced an analytical portion that was sufficiently homogeneous to ensure that the sub-sampling uncertainty was acceptable. Codex Guideline CAC/GL 59-2006 on the estimation of uncertainty of results provides the statistical background and the principle of estimating sample processing from the spiking experiments.

The first approach involved analysing, in a single batch, all analytical portions deriving from a naturally contaminated sample. In this case the variability deriving from the analysis of pesticide residues in the sample approximates the variability of sample processing as all other factors are kept constant.

The second approach consisted of evaluating differences arising from spiking experiments using blanks. Pesticide-free vine leaves were spiked before sample processing and the results were compared to those generated by spiking individual analytical portions after homogenization. In this case, the difference between the two procedures approximates the variability arising from sample processing, since all factors were kept as constant as possible during the analytical procedure.

The naturally contaminated sample used to study the sample processing homogeneity according to the first approach was found to contain chlorpyrifos residues, so chlopyrifos was selected as the study pesticide for the spiking experiments in the second approach.

The preliminary results of the sample processing experiments showed that both approaches accounted for less than 10% of the variability deriving from the sample processing step. This result confirms that cryogenic processing of vine leaves by the method applied, using liquid nitrogen with a mortar and pestle, gives an acceptably homogeneous sample, and that the sub-sampling variability is within acceptable levels.This aspect of the methodology is, therefore, fit-for-purpose for regulatory testing.

Coordinated Research

In 2018, the FEPL coordinated and provided technical input to two coordinated research projects (CRPs) in the fields of food authenticity and traceability and initiated one new project.

Accessible Technologies for the Verification of Origin of Dairy Products as an Example Control System to Enhance Global Trade and Food Safety

The coordinated research project on "Accessible Technologies for the Verification of Origin of Dairy Products", which concluded in 2018, successfully demonstrated the feasibility of using stable isotope and trace element (SITE) analysis, combined with other nuclear and related techniques, to establish geographical origin and authenticity of liquid milk and powdered milk produced in Member States. The project also enhanced analytical capabilities for food authenticity testing and food traceability system verification and raised awareness in Member States of SITE analysis and its wider applications to food traceability (production methods and geographical origin) and authenticity, as well as its potential to reduce barriers to trade and enhance consumer confidence. The final research coordination meeting (RCM) concluded with a 1-day stakeholder workshop for policy-makers, the dairy industry, retailers and academics to raise awareness of the methods developed and research outputs. Over the 5-years of the project more than 160 outputs were produced by 10 contract holders covering the categories of scientific journal publications (27), oral presentations (21), poster communications (23), PhD studentships (8), MSc studentships (8), undergraduate projects (4), trained personnel (28), new links to industry (10), new academic collaborations (14) and standard operating procedures (19).

Field deployable analytical methods to assess the authenticity, safety and quality of food

The coordinated research project on 'Field-deployable analytical methods to assess the authenticity, safety and quality of food' is a joint project between FEPL and the IAEA's Nuclear Science and Instrumentation Laboratory (NSIL), which aims to close the gap between instrumental capabilities found in research labs and technologies that can be easily used by various national gate-keepers in developing countries, such as national customs authorities and food regulators. The project's second RCM was held in December 2018. The consortium has made good progress with developing methods for hand-held and portable spectroscopic devices such as near infra-red (NIR), Fourier transform-infrared (FT-IR) and gas chromatography-mass spectrometry (GC-MS). Two papers, 'A Laser Ablation Resonance Ionisation Mass Spectrometer (LA-RIMS) for detection of isotope ratios of uranium at ultra-trace concentrations from solid particles and solutions' and 'Atmospheric Pressure Chemical Ionisation (APCI) and Photoionisation (APPI) mass spectrometry for rapid detection of adulteration of vegetable oils', have been submitted to the Journal of Analytical Atomic Spectrometry (JAAS) and the

Journal of Agricultural and Food Chemistry (JAFC), respectively. The consortium is also planning an inter-laboratory comparison exercise with the low-cost molecular sensor from SCIO on the authentication of oregano spice. SCIO units have been distributed to all project participants from developing countries along with protocols. FEPL has also developed SOPs for the SCIO to detect the presence of carcinogenic formaldehyde preservative in milk.

Implementation of Nuclear Techniques for AuthentiCaTion of Foods with High-Value Labelling Claims (INTACT Food)

A new coordinated research project was conceived by FEPL staff, elaborated through a consultants' meeting in 2018 and subsequently approved to commence in 2019.

Numerous foods are sold at premium prices because of high-value labelling claims related to specific production methods, unique characteristics and origins. These claims include agricultural, geographic, cultural, ethical and nutraceutical labelling specifications that add value to the products. In order to protect consumers from fraud and potential unintended food safety issues, analytical methods are required to confirm such claims. Several nuclear, isotopic and related techniques have proven suitable for confirming a wide range of high-value labelling claims such as free-range, organic, natural/synthetic, etc. The overall objective of the approved CRP is to enable developing countries to protect and promote food products with high-value labelling claims by development and application of nuclear and related techniques. The project thereby aims to safeguard consumers and reputable producers; ensure regulatory, religious and ethical compliance; stimulate domestic markets; etc.

EU Projects

FoodIntegrity

'FoodIntegrity' is the short title of the EU-funded Seventh Framework Integrated Project 'Ensuring the Integrity of the European food chain' that started in January 2014 and finished in December 2018. The final meeting of the project consortium and the associated 5th international conference took place in, Nantes, Frances on the 13th and 14th to 15th November 2018, respectively. Mr. Simon Kelly (FEPL) was the workpackage 1 (Food Integrity Network) leader and the FEPL actively participated in workpackage 2 (Knowledge Base); workpackage 10 (Industrial Integration) and workpackage 11 (Dissemination and Training). The main aim of the project was to build capabilities to fight food fraud and to ensure the authenticity, safety and quality of European food and the integrity of its supply chains. It involved producers, industry, retailers, public administrators, control bodies, NGOs, analytical laboratories and researchers. Results included a 'Food Integrity Knowledge Base' of food authenticity issues and methodologies, which will be hosted by the EU's Directorate General – Joint Research Centre Food Fraud Unit in Geel, Belgium. A series of scientific opinions (SOs) compiled by workpackage 1, on a range of topics related to food authenticity and fraud detection, is being published in the peer-reviewed journal Trends in Food Science & Technology. These scientific opinions will also be followed up with videos and info-graphics to promote their open-access, which are available on the FoodIntegrity Project YouTube channel https://www.youtube.com/channel/UCEBrY7D_LGFc_JkHQBzAddg

Authent-Net

Authent-Net (http://www.authent-net.eu/) was a two-year Horizon 2020 project, completed in 2018, designed to facilitate sustainable cooperation between national and international research funding bodies in the area of food authenticity, and to improve the competitiveness of the food supply chain and the consumer confidence in it by means of better-coordinated, cost-effective R&D. The Authent-Net consortium consisted of 19 partners from 12 countries. As a partner in the project, FEPL provided a more international dimension to the European partners. Outputs of the project include the web-based Food Authenticity Research Network Hub (FARNHub, http://farnhub.authent.cra.wallonie.be/) which provides registered users with an overview of currently available resources related to food

authenticity, and the Authent-NET website and communication tools (http://www.authent-net.eu). Another major output is the low-level European voluntary standard CEN (Comité Européen de Normalisation) Workshop Agreement CWA 17369:2019, entitled 'Authenticity and fraud in the feed and food chain - Concepts, terms, and definitions', which was published on 23 January 2019.

Dissemination of Research Results

The results of the research and the methods developed or adapted and validated in the FEPL are made available to Member States through various mechanisms, including training courses, workshops, publications in the scientific literature and via the internet, public outreach events, conferences and symposia. The 'Food Contaminant and Residue Information System' (FCRIS, http://nucleus.iaea.org/fcris/) provides useful data on food contaminants and residues and includes analytical methods databases. 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

Twelfth European Pesticide Residue Workshop (EPRW) Munich, Germany, 21 to 25 May 2018. The EPRW is recognized worldwide as a platform that covers the latest concepts and developments in the field of pesticides analysis in food and beverages. The 12th EPRW was attended by more than 560 participants from more than 42 countries. Topics included: pesticides applied according to good agricultural practices; risk analysis, including communication; environmental contamination as a route of entry of pesticides to food and feed; residues in organic production; pesticide-relevant compounds; pesticide residue analytical technology (including sample processing); and high-tech instrumentation and regulations for maximum residue limits. A total of 30 plenary lectures and 202 posters were presented, including two posters presented by Ms. Britt Maestroni (FEPL), 'Assessment of the withholding period for organophosphorus pesticides applied to vine leaves' and 'Networking strategies to ensure food safety and environmental quality in Latin America and the Caribbean', both authored by FEPL staff and collaborating scientists.

The Belfast Summit on Global Food Integrity (ASSET 2018), Belfast, UK, 28-31 May 2018. ASSET 2018 brought together approximately 600 food safety and security experts from 48 countries, spanning academia, industry, agriculture, NGOs and regulators. Experts and participants at this high-level, international Summit discussed the problems of feeding a growing population whilst maintaining the integrity of the food supply, considering issues such as pollution, climate change, food fraud and food terrorism. Mr. Andrew Cannavan (FEPL) chaired a panel discussion session on the state of the art in the control of food fraud, which focused on understanding the growing threat to the integrity of the global food system from food fraud and food terrorism. Mr. Cannavan also presented two posters on research performed in the FEPL; 'Rapid screening techniques for extra virgin olive oil authentication' (which was awarded a poster prize) and 'Rapid isotope analysis of non-exchangeable hydrogen in sugar molecules derivatised with MBTFA, using GC-chromium/high-temperature-conversion-IRMS'.

Second International MoniQA Symposium on Food Fraud Prevention and Effective Food Allergen Management, Vienna, Austria, 7-8 June 2018. MoniQA, the International Association for Monitoring and Quality Assurance in the Total Food Supply Chain, was launched in 2011 as a result of a successful EU-funded networking project. It is an international and interdisciplinary network of professionals from institutions working in food research, regulatory bodies and trade, providing solutions to promote a safer and secure food supply, worldwide. The 2nd International MoniQA Symposium attracted 77 delegates from 18 countries, representing all aspects of the agrifood sector. Mr. Andrew Cannavan (FEPL) gave the keynote address, discussing the global perspective of food fraud and a 'systems' approach to dealing with it, with examples drawn from the Joint FAO/IAEA Division's international research projects and capacity building in the developing world.

Metabolomics 2018 – 14th Annual Conference of the Metabolomics Society, Seattle, Washington, USA, 24-28 June 2018. The Metabolomics 2018 programme covered the full range of metabolomics research topics, with major scientific themes of system biology, big data, technology advances, translational science, plant metabolomics, the microbiome and the exposome, including environmental and nutritional metabolomics, which were reflected in the diverse parallel scientific sessions. The conference had more than 200 participants from more than 20 countries. Ms. Zora Jandrić (FEPL) gave an oral presentation on applied research performed in FEPL on a comprehensive strategy for identification of food origin using fast screening techniques and high-resolution mass spectrometric metabolomics methods to support FAO/IAEA Member States in ensuring sustainable food systems.

Fifth International 'FoodIntegrity' Conference, Nantes, France, 14-15 November 2018. The theme for the conference was 'Delivering real world solutions'. The conference included parallel sessions on 'Complex foods: tools for food authenticity assessment', 'The lab comes to the factory', 'Transparency and trust in the food chain', 'Standardisation: new initiatives', 'Molecular biology approaches to food integrity', 'Available IT tools using data for food authentication', 'Organic food authentication', 'Industry are the main victims of food fraud? A debate on a new paradigm', 'Targeted versus non-targeted: problems and solutions' and 'Will Blockchain really solve our food fraud problems?'. Mr. Simon Kelly (FEPL) participated in the organisation and implementation of the parallel session focusing on the authenticity of organic foods and gave an oral-presentation entitled 'Is it organic? What do existing analytical techniques have to offer and how close are we to implementing them?'.

The Long Night of Research, Vienna International Centre, Vienna, Austria, 13 April 2018

The Long Night of Research (Lange Nacht der Forschung) is an Austria-wide event held every two years and coordinated by several Austrian government ministries, that aims to spark interest in science and research. In 2018, Vienna International Centre (VIC) opened its doors for this event for the second time, to participate in the eighth Long Night of Research. The VIC was one of around 250 exhibit locations across the country and had approximately 1600 visitors to the exhibition.

From 5 pm until midnight, stations and displays in the VIC Rotunda showcased the science and research of the IAEA and several other UN organisations to the general public. IAEA scientists hosted more than a dozen exhibition booths, including displays by the five laboratories of the Joint FAO/IAEA Division. The Food and Environmental Protection Laboratory (FEPL) exhibition booth focused on testing for food authenticity, posing the question 'is my food safe and am I getting what I paid for?'. Eight members of the FEPL team manned the booth, providing information to the visitors and giving hands-on demonstrations of hand-held and bench-top spectrophotometric instruments for which applications are being developed in FEPL to provide screening tests for the authenticity of foods, or detection of adulteration.

The FEPL team at their Long Night of Research exhibition booth

CAPACITY BUILDING

The FEPL provided technical management for twenty-two national and five regional technical cooperation projects in 2018. Analytical methods and technology packages were transferred and applied through training workshops held in Member States or at Seibersdorf and fellowships in, or scientific visits to, our laboratories in Seibersdorf. Human resource capability was enhanced through the training of 418 scientists, analytical chemists, laboratory personnel and food inspectors via 16 courses, workshops or seminars.

The first Coordination Meeting of the Regional Cooperative Agreement project 'Enhancing Food Safety and Supporting Regional Authentication of Foodstuffs through Implementation of Nuclear Techniques' (RAS 5081) was held in Vienna, 5-9 February 2018. It was attended by 18 representatives from 18 participating countries: Australia, Bangladesh, Cambodia, China, Fiji, India, Indonesia, Lao P.D.R., Malaysia, Mongolia, Myanmar, Nepal, New Zealand, Philippines, Singapore, Sri Lanka, Thailand and Vietnam.

Staff from the FEPL contributed directly to capacity building through a number of expert missions to Member States, including Bangladesh, China, the Dominican Republic, Montenegro, Namibia, Panama, South Africa and Viet Nam. The training activities and meetings provided a platform for interdisciplinary networking between stakeholders in the "farm-to-fork" food chain and fostered the formation of a global network.

Training course on 'Fundamentals of Using Nuclear Techniques for Verifying Food Authenticity'

The first training course of the regional technical cooperation project 'Enhancing Food Safety and Supporting Regional Authentication of Foodstuffs through Implementation of Nuclear Techniques' (RAS 5081) took place at the University of Otago, Dunedin, New Zealand, 25 June-6 July 2018. It was attended by 22 participants from 12 Member States; Bangladesh, Cambodia, Fiji, Indonesia, Korea, Lao P.D.R., Mongolia, Myanmar, Nepal, Sri Lanka, Thailand and Vietnam. The Host Country Organizer was Mr. Russell Frew (University of Otago, New Zealand) and the expert trainers were Mr. Kiri McComb (University of Otago, New Zealand), Ms Karyne Rogers (Geological and Nuclear Sciences, New Zealand) and Mr. Simon Kelly (FEPL). The overall aim of the two-week course was for the participants to gain a basic knowledge of, and experience in, the main nuclear and complementary techniques used to confirm the authenticity and origin of foodstuffs. The course provided theoretical and hands-on training in isotope ratio mass spectrometry (IRMS); atomic absorption spectrophotometry (AAS); inductively coupled plasma - mass spectrometry (ICP-MS) and infrared spectroscopy. The course consisted of whole-group lectures and laboratory sessions on a small-group rotational basis.

'FoodIntegrity' Training and Exchange Programme

A training and exchange programme was implemented as one of the activities of the project 'FoodIntegrity: Assuring quality and authenticity in the food chain', which was funded under the European Union's Seventh Framework Programme. Under this programme, FEPL held two training courses on analytical methodology for verification of the authenticity of food, one on metabolomics and the other on stable isotope analysis.

Metabolomics for verifying the authenticity of food, FEPL, Seibersdorf, Austria, 4-8 June 2018

This training workshop was attended by 5 scientists from institutes in Belgium, the Czech Republic, Indonesia, Spain and UK. The purpose of the workshop was to introduce untargeted and targeted metabolomics approaches and share knowledge on their application for food authentication, and to discuss the current limitations and future potential of the techniques. The course included hands-on training in the laboratory and on data analysis using sophisticated statistical tools, e.g. principle components analysis (PCA), (orthogonal) partial least squares discriminant analysis ((O)PLS-DA), soft

independent modelling by class analogy (SIMCA), data-driven (DD)-SIMCA, one class classification, and others. The training course generated a great deal of interest and interaction, information and knowledge exchange, and was an excellent opportunity to raise awareness of the benefits and challenges of a metabolomics approach for food authenticity and safety applications.

Stable isotopes for verifying the authenticity of food, FEPL, Seibersdorf, Austria, 27-31 August 2018

The training workshop on stable isotope analysis for verifying the authenticity of food was attended by 4 scientists from institutes in Denmark, Malaysia, Poland and the UK. The 5-day course included lectures and hands-on laboratory sessions covering an introduction to food fraud and the application of isotope ratio mass spectrometry (IRMS); setting up the elemental analyser (EA) and IRMS for analysis; routine EA-IRMS operation, fault finding and maintenance; preparation of honey protein following the Association of Official Analytical Communities (AOAC) methodology; calibration, data processing and analysis; quality control, proficiency testing and ion-source dismantling and cleaning.

The quality of the training provided by FEPL was independently assessed by the Food Integrity Project work package 11 'dissemination' team and received an overall 'excellent' rating by the attendees.

RALACA Laboratory Network

The Red de Latino America y el Caribe (RALACA) is a non-profit network that brings together analytical laboratories to enhance regional capabilities for food safety and environmental sustainability. RALACA was established with assistance and guidance from FEPL, and Ms. Britt Maestroni serves on the governing board of the network, as well as taking an active role in the network's activities. The main objective of RALACA is to strengthen the technical capabilities of the laboratories in the region, promote scientific cooperation among the countries involved in the network, and foster communication between all national stakeholders, including decision makers. Information sharing is key to enhancing regional opportunities. Meetings are held regularly either online, through webinars, or as side events of technical meetings and/or training events. To date, RALACA comprises 56 institutions in 21 countries.

Since its inception, RALACA has published, through its associated members, 29 articles in scientific journals, 74 posters in scientific conferences, 32 oral presentations, 7 book chapters and 16 analytical methods for food safety. RALACA has also organized and held 26 meetings with decision makers, participated in 14 radio interventions and 5 TV programmes and prepared 10 brochures. RALACA presents monthly webinars on a range of food safety-related topics. The presentations are available on the RALACA web site at: http://www.red-ralaca.net/e-learning-2. The first issue of the RALACA newsletter (August 2018) can be accessed at http://www.red-ralaca.net/images/pdfs/Newsletter_Vol_1.pdf.

Field Missions

In addition to the training courses and workshops, FEPL staff carried out several missions to Member States to provide technical assistance, policy advice and project planning.

Enhancing food safety in Namibia

Ms. Britt Maestroni (FEPL) visited project NAM5015 counterparts in Walvis Bay and Windhoek from 16-24 February 2018 to revise and refine the project work plan. The project counterparts are the

Regulatory and Consumer Protection Business Unit of the Namibian Standards Institution (NSI) and the Food safety and Standards Unit of the Agro-Marketing and Trade Agency (AMTA). The focus of the project is on strengthening sampling and inspection, with possible accreditation; enhancement of the laboratory capacity for heavy metals measurements in fishery and poultry products (NSI) and mycotoxins in agricultural produce (AMTA); and to collect existing analytical data and carry out a first risk assessment for cadmium in oysters (NSI) and mycotoxins in grains (AMTA) in 2019.

Developing indicators to determine the effect of pesticides, heavy metals and emerging contaminants on continental aquatic ecosystems important to agriculture and agroindustry - final project meeting

Ms. Britt Maestroni participated in the final coordination meeting of project RLA7019 from 5-9 March 2018 in Santa Clara, Panama. The meeting assessed project outputs and outcomes, identified lessons learned and future challenges and drafted a project report. The meeting had 31 participants from 12 countries. The RALACA network of laboratories was enhanced within the framework of this project and it will play a vital role in ensuring sustainability of analytical activities and help the transfer of information, analytical tools and methodologies to the entire Latin American and Caribbean region.

Building food safety capacity in Bangladesh

Mr. Simon Kelly (FEPL) visited the Bangladesh Atomic Energy Commission (BAEC) Laboratories in Dhaka, Bangladesh, from 20-26 April 2018 to finalise the work plan and activities for the technical cooperation project 'Building Capacity in Improving Food Safety Using Nuclear and Other Complementary Analytical Techniques' (BGD5032). Mr. Kelly also had meetings with collaborating groups at the Department of Genetic Engineering and Biotechnology, (Dhaka University) and the BAEC Research Reactor team for discussion on its previous and potential use for food authentication studies e.g. neutron activation analysis.

Food Integrity session of the African Food Safety Workshop

Mr. Simon Kelly gave an oral presentation on the use of stable isotope and trace element (SITE) profiling to confirm the authenticity and origin of food during the Food Integrity session of the African Food Safety Workshop held in Pretoria, South Africa, 4-8 June 2018.

Improving pollution management of persistent organic pollutants in Latin America

Ms. Britt Maestroni travelled to Santo Domingo, Dominican Republic, from 4-8 June 2018, to participate in the intermediate coordination meeting of IAEA technical cooperation project RLA5059 on 'Improving Pollution Management of Persistent Organic Pollutants to Reduce the Impact on People and the Environment'. The meeting was attended by 16 participants from 13 countries. Ms. Maestroni also gave a lecture on food safety to about 40 students at the local university.

Technical assistance to the Centre for Eco-Toxicological Research (CETI), Podgorica, Montenegro

Ms. Zora Jandrić (FEPL) visited CETI under technical cooperation project MNE5004, 'Strengthening Technical and Institutional Capacities of the National Reference Laboratory for Food and Feed Control', to assess and verify the laboratory conditions for sample preparation and hosting a liquid chromatography - tandem mass spectrometry system (LC-MSMS).

Verifying food authenticity in China

Verifying food authenticity is a high priority for China; not only to protect consumers from fraud, but also to protect them from unintended food safety issues that are derived from clandestine food production activities in unlicensed or unsanitary conditions. The FEPL has initiated a collaboration with

the Institute of Quality and Standards for Agricultural Products (IQSAP), Zhejiang Academy of Agricultural Sciences (ZAAS), to develop systems utilising advanced stable isotope techniques and an integrated and multidisciplinary approach, to confirm the authenticity and origin of Chinese agro-products. Mr. Simon Kelly (FEPL) visited IQSAP from 15-19 October 2018 to discuss and review their activities in food authenticity and traceability.

Promoting interlaboratory comparison and accreditation for food safety testing in Vietnam

Ms. Britt Maestroni (FEPL) undertook a mission to Vietnam from 15-18 October 2018 to discuss all aspects related to the implementation of IAEA technical cooperation project VIE5022, 'Promoting Interlaboratory Comparison and Accreditation in Testing Chemical Contamination for Food Safety' and to assess the current laboratory capacity of Quatest 3, located in Bien Hoa, Vietnam. The focus of the project is on mycotoxin contamination of food products.

Association of Analytical Communities (AOAC) Sub-Saharan Africa Section inaugural meeting

The inaugural meeting of the AOAC Sub-Saharan Africa Section was held in Pretoria, South Africa, 5-7 November 2018. The Section represents 46 African countries, creating an Africa-based platform to improve the standard and performance of analytical science within the region. The meeting had more than 100 participants, mainly from across Africa. Mr. Andrew Cannavan (FEPL) participated in a high-level dialogue to outline the economic imperative for food safety capacity building and its role as a prerequisite for trade and economic development.

Support for the development and application of international standards

The FEPL contributed to the publication of three Scientific Opinions (SO) in 2018 through participation in the EU 7th Framework Integrated Project "FoodIntegrity: Ensuring the Integrity of the European food chain". The SOs are available in open-access format at the following links:

- SO1: Stable isotope techniques for verifying the declared geographical origin of food in legal cases https://www.sciencedirect.com/science/article/pii/S0924224416302771
- SO3A: What are the scientific challenges in moving from targeted to non-targeted methods for food fraud testing and how can they be addressed? – Spectroscopy case study https://www.sciencedirect.com/science/article/pii/S0924224417307938
- SO3B: The scientific challenges in moving from targeted to non-targeted mass spectrometric methods for food fraud analysis: A proposed validation workflow to bring about a harmonized approach
  https://www.sciencedirect.com/science/article/pii/S0924224418302449

The FEPL also contributed to the formulation of the European Committee for Standardization (CEN) Workshop Agreement CWA 17369:2019, 'Authenticity and fraud in the feed and food chain – Concepts, terms, and definitions' officially published by CEN on the 23rd January 2019. This is an important food authenticity terminology standard that should eventually become a full ISO standard.

The Joint FAO/IAEA Division participates in the Codex Alimentarius Commission and various committees to provide support the development and application of international guidelines and standards. In 2018, Mr. Simon Kelly (FEPL) represented the Joint Division at the Codex Committee on Methods of Analysis and Sampling, while Mr. Andrew Cannavan participated in the 41st Codex Alimentarius Commission meeting.

Integrated analytical approaches for pesticide management

Two books, summarising the results of an inter-regional project run by FEPL using various funding mechanisms were published by Elsevier in 2018. The book, 'Integrated analytical approaches for pesticide management' collated inputs from 26 institutes in 12 countries, as well as FEPL, to provide generic guidelines on pesticide analysis and environmental monitoring. The second book, a laboratory manual entitled, "Analytical methods for agricultural contaminants", comprises operating procedures

for 30 analytical methods from 17 institutes in 7 countries and FEPL. The books demonstrated the success of the project in participating countries and will allow take-up of the methodology by other countries. A template for data management and communication of results to decision-makers was also developed and is available on the RALACA website (red-ralaca.net).

Advice and Information Exchange

Staff of the FEPL were members of the scientific or organising committees of three international conferences in 2018: the Eighth International Symposium on Hormone and Veterinary Drug Residue Analysis, Ghent, Belgium, 22-25 May 2018; the Belfast Summit on Global Food Integrity, Belfast, UK, 28-31 May 2018; and the 9th European Food Safety & Standards Conference, Dublin, Ireland, 29-30 November 2018. In 2018, FEPL staff also contributed to the committees for future international conferences: IUPAC 2019 - 14th International Congress of Crop Protection Chemistry, Ghent, Belgium, 19-24 May 2019; and the 9th EuroResidue Conference on residues of Veterinary Drugs in Food, Egmond aan Zee, The Netherlands, May 2020.

FEPL staff regularly provide advice through participation as members of, for example, the UK's Food Authenticity Methodology Working Group, the advisory board of the ASSET (Assured, Safe and Traceable Food) Centre at Queen's University Belfast, UK, the Global Food Safety Partnership, and the Institute of Food Science and Technology.

Fellowships, Scientific Visitors and Interns

Name	Country	Status	Duration	Topic
Ahmad Hassali, Hazlina	Malaysia	Scientific visit	1 week	Metabolomics and chemometrics for food authenticity testing
Hussan, Husham Nasreldin Mustafa Hussan	Sudan	Scientific visit	1 week	Analysis of pesticides in agricultural products.
Jahajha, Ali	Palestine	Fellow	3 months	Multi-analyte analysis of chemical residues in honey, method validation
Jin, Shunru	China	Cost-free Fellow	12 Months	Analytical methods for food control
Nassar, Bashaer	Palestine	Fellow	3 months	Multi-analyte analysis of chemical residues in honey, method validation
Vaughan, Amber	UK	Intern	12 months	Food authenticity testing using stable isotope and spectroscopic analytical techniques
Xu, Xiao	China	Intern	3 months	Analytical methods for food control

PUBLICATIONS

CACERES, T., MAESTRONI, B., ISLAM, M., CANNAVAN, A. (2018). Sorption of 14C-carbofuran in Austrian soils: evaluation of fate and transport of carbofuran in temperate regions. Environmental Science and Pollution Research Journal, 26: 986-990.

MAESTRONI, B., GHANEM, I., CORRELL, R., ABU ALNASER, A., ISLAM, M., CESIO, V., HEINZEN, H., CANNAVAN, A. (2018). Required withholding period for vine leaves following spraying with pesticide. Journal of Health and Environmental Research, 4: 140-152.

KELLY, S. (2018) Is it Organic? What do existing analytical techniques have to offer and how close are we to implementing them? Book of abstracts of the 5th FoodIntegrity Conference, Assuring the integrity of the food chain: delivering real world solutions, Nantes, France, 14-15 November 2018, 94.

PÖPPING, B., BUCK, N., BANATI, D., BRERETON, P., CALANCHA, M., GADANHO, M., GENDEL, S., KELLY, S., LESEUR, C., MENZEL, M., MORLING, A., SANER, S., SPINK, J., WIGGERS, V.-D. (2018). Guidance document towards food authenticity, an initiative of ILSI Europe. Book of abstracts of the 5th FoodIntegrity Conference, Assuring the integrity of the food chain: delivering real world solutions, Nantes, France, 14-15 November 2018, 75.

MAESTRONI, B., FERRIS, I.G., CORRELL, R., KOHLMANN, B., NORDGAARD, A., KOOKANA, R.S., CANNAVAN, A. (2018). Generic guidelines on integrated analytical approaches to assess indicators of pesticide management practices at a catchment scale. Black-box monitoring and the laboratory's role in fostering good agricultural practice. In *Integrated Analytical Approaches for Pesticide Management*, Eds. Maestroni, B., Cannavan, A. Academic Press, London, UK, pp 7-25.

MABIT, L., FULAJTAR, E., TOLOZA, A., OCHOA, V., MAESTRONI, B. (2018). Implementation and optimization of soil sampling: some practical guidance and considerations. In *Integrated Analytical Approaches for Pesticide Management*, Eds. Maestroni, B., Cannavan, A. Academic Press, London, UK, pp 47-61.

OCHOA, V., MAESTRONI, B. (2018). Pesticides in water, soil and sediments. In *Integrated Analytical Approaches for Pesticide Management*, Eds. Maestroni, B., Cannavan, A. Academic Press, London, UK, pp 133-145.

MAESTRONI, B., CANNAVAN, A. Eds. (2018). *Integrated Analytical Approaches for Pesticide Management*. Academic Press, London, UK. ISBN: 9780128161555.

JANDRIC, Z., CENTONZE, V., ISLAM, M., CANNAVAN, A., FREW, R. (2018). A comprehensive strategy for identification of food origin. Book of abstracts of Metabolomics 2018: 14th Annual Conference of the Metabolomics Society, Seattle, Washington, 24-28 June 2018, 19D.

KELLY, S., BRODIE, C., HILKERT, A. (2018). Isotopic-Spectroscopic Technique: Stable Isotope-Ratio Mass Spectrometry (IRMS). In *Modern Techniques for Food Authentication*, Ed. D-W. Sun. Academic Press, MA, USA, pp. 349-413.

MAESTRONI, B. (2018). General requirements for food safety analysis. In *Analytical Methods for Agricultural Contaminants*, Eds. Maestroni, B., Ochoa, V., Cannavan, A. Academic Press, London, UK, pp 5-22.

MAESTRONI, B., OCHOA, V., CANNAVAN, A. Eds. (2018). *Analytical Methods for Agricultural Contaminants*. Academic Press, London, UK. ISBN: 9780128159408.

MAESTRONI, B., VAZQUEZ, A.R., AVOSSA, V., GOOS, P., CESIO, V., HEINZEN, H., RIENER, J., CANNAVAN, A. (2018). Ruggedness testing of an analytical method for pesticide residues in potato. Accreditation and Quality Assurance, 23: 303-316. Available online at https://doi.org/10.1007/s00769-018-1335-7.

CANNAVAN, A. (2018). The global perspective of food fraud. Quality Assurance and Safety of Crops and Food, 10, supplement 1 – Food Fraud, 2nd International MoniQA Symposium on Food Fraud Prevention and Effective Food Allergen Management, Vienna-Vösendorf, Austria, 7-8 June 2018, S3.

MAESTRONI, B., ABU ALNASER, A., GHANEM, I., ISLAM, M., CESIO, V., HEINZEN, H., KELLY, S., CANNAVAN, A. (2018). Validation of an analytical method for the determination of pesticide residues in vine leaves by GC-MS/MS. Journal of Agricultural and Food Chemistry, 66: 6421-6430.

CANNAVAN, A., JANDRIC, Z., ISLAM, M., KELLY, S. (2018). Rapid screening techniques for extra virgin olive oil authentication. Book of abstracts of the Belfast Summit on Global Food Integrity (ASSET 2018), Belfast, UK, 28-31 May 2018, 131.

CANNAVAN, A., ABRAHIM, A., KELLY, S. (2018). Rapid isotope analysis of non-exchangeable hydrogen in sugar molecules derivatised with MBTFA, using GC-chromium/high-temperature-conversion-IRMS. Book of abstracts of the Belfast Summit on Global Food Integrity (ASSET 2018), Belfast, UK, 28-31 May 2018, 179.

MAESTRONI, B., GHANEM, I., CORRELL, R., ALNASER, A., ISLAM, M., CESIO, V., HEINZEN, H., CANNAVAN, A. (2018). Assessment of the withholding period for organophosphorus pesticides applied to vine leaves. Book of abstracts of the 12th European Pesticide Residue Workshop, Munich, Germany, 21-25 May 2018, 204.

LOEWY, M., NARIO, A., CARAZO, E., MASIS, M., MAESTRONI, B. (2018). Networking strategies to ensure food safety and environmental quality in Latin America and the Caribbean. Book of abstracts of the 12th European Pesticide Residue Workshop, Munich, Germany, 21-25 May 2018, 267.

EXTRA-BUDGETARY SUPPORT

THE INSECT PEST CONTROL LABORATORY

EXECUTIVE SUMMARY

In the Livestock Pest (LP) group of the Insect Pest Control Laboratory (IPCL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, an assessment was made of the effect of irradiation on the vectorial capacity of sterile males for trypanosomes. The initial results of this work carried out in collaboration with the Institute of Tropical Medicine in Antwerp, Belgium, indicate that an irradiation treatment of 110 Gy reduced the trypanosome infection rate in salivary glands and the midgut as compared to non-treated flies. The impact of irradiation was likewise assessed on the profile of cuticular hydrocarbons (CHC). Irradiating *Glossina morsitans morsitans* males with doses of 20, 50 and 110 Gy had no effect on the CHC profile.

Sterile male tsetse flies that are used in SIT programmes are often not 100% sterile, and concerns have been voiced on the potential of introducing new genetic material in the target area when the released strain does not originate from the release area. Initial results indicate that productivity of females mated with F_1 males (descendants of crosses where one parent was irradiated) was not affected by irradiation dose, but the productivity of F_1 females mated with untreated males was significantly reduced with increasing dose used for the parents.

In the Plant Pest (PP) group, work continued under the FAO/IAEA/USDA agreement on Development of Phytosanitary and Regulatory Treatments for Exotic Tephritid Fruit Flies. Work was carried out to assess the effect of hypoxic and severe-hypoxic conditioning before and during irradiation of fruits infested with fruit flies. Preliminary results indicate that hypoxia can increase the survival of *Anastrepha fraterculus* (Brazilian-1), *Anastrepha ludens*, *Bactrocera dorsalis*, and *Ceratitis capitata* at low doses of gamma radiation. At high doses of irradiation, low-oxygen conditioning treatments did not increase the survival rates of any fruit fly species evaluated.

Work continued as well on the development of the SIT package for *Drosophila suzukii*. Simulation experiments of transport conditions were carried out to assess the effect of cold treatment and/or hypoxia on the quality of irradiated pupae. It was shown that cold treatment of pupae in combination with hypoxia seemed to be a promising method to avoid emergence of flies while preserving their quality. Experiments were likewise carried out to assess the competitiveness of irradiated *D. suzukii* in small laboratory cages. Preliminary results indicate that irradiated males were less successful in mating with the females as compared with fertile males, but males irradiated under hypoxia conditions achieved more matings than sterile males irradiated under normoxia conditions.

In the Human Disease Vectors (HDV) group, work continued on the development of more efficient rearing for mosquitoes. In that respect, an accurate larval counter was developed and tested and a new larval diet was developed and tested with one of the more expensive ingredients (bovine liver powder) replaced with proteins from black soldier flies (the diet was 80% cheaper than the standard diet). Development of a new blood feeding system using sausage casing with heating rods took place, and the flight ability device that was developed to assess the quality of *Aedes* mosquitoes was adapted for use with *Anopheles arabiensis,* the vector of malaria. Further work has focussed on the effects of irradiation in relation to pupal age and pupal size.

In the Genetics and Molecular Biology (GMB) group of the IPCL, recombination frequencies in *Aedes* mosquitoes between various mutations and the M locus were assessed. The currently available data show that the recombination frequencies show transgenerational stability and do not seem to be influenced by the age of the adults. On the other hand, these recombination frequencies need to be reduced or even better, eliminated, to be effectively incorporated in a genetic sexing strategy. Further work was carried out on an assessment of appropriate inversions that could reduce or even eliminate recombination between the two loci of interest (M locus and selectable marker) and provide a GSS of

higher genetic stability (2nd generation GSS). In addition, work was carried out on the genetic changes in fruit flies subjected to several generations of domestication.

In 2018, staff of the IPCL published 51 scientific papers in peer reviewed journals, either as the lead author or as a co-author.

In 2018, the IPCL hosted 14 cost-free experts (CFE) and 9 consultants (C) (of which 5 were PhD students), 7 interns, 8 fellows (F) and 3 scientific visitors (SV).

The GMB and PP groups carried out 64 fruit fly shipments to 18 institutions (in Senegal, Greece, Germany, Italy, Bangladesh, Uruguay, Czech Republic, Pakistan, Brazil, Sweden, UK, Spain, Kenya, Mauritius, Mexico and France), and 2 shipments of preserved fruit flies to 2 institutions (in Italy and Belgium). The LP group carried out 148 tsetse shipments of 270,407 pupae (239,744 *G. palpalis gambiensis* to Senegal) to 9 institutions in 8 countries (Senegal, France, Uganda, USA, Belgium, Zimbabwe, South Africa and Netherlands). The HDV group carried out 14 mosquito shipments to 8 institutions (in Spain, Sweden, Brazil, Germany, Mexico, Austria, France and Switzerland).

In 2018, the IPCL received 672 visitors from 88 countries.

STAFF

Name	Title
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Bourtzis, Kostas	Molecular Biologist/Geneticist
Bouyer, Jeremy	Entomologist (Human Disease Vectors)
Caceres, Carlos	Entomologist (Plant Pests)
Parker, Andrew	Entomologist (Livestock Pests)
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Cancio Martinez, Elena	Laboratory Technician
Dammalage, Thilakasiri	Laboratory Technician
Pillwax, Gulizar	Laboratory Technician
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Wallner, Thomas	Laboratory Technician
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Beckham, Stephanie	Programme Assistant
Wimberger, Tamara	Team Assistant

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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Livestock Pests

Impact of ionizing radiation on the vectorial capacity of tsetse flies for trypanosomes

Tsetse flies are competent vectors of trypanosomes, the causative agents of sleeping sickness in humans and nagana in animals. Despite the decrease in prevalence of human sleeping sickness in the last decade, nagana remains a serious problem for agriculture in much of sub-Saharan Africa.

Sterile tsetse fly males are blood-feeding which makes them a potential vector when released in large quantities in a target area that is under SIT implementation. The risk of disease transmission must be avoided completely in areas endemic for human sleeping sickness. The impact of irradiation on the vectorial capacity of sterile males remains poorly understood, therefore an assessment was made on the vectorial capacity of the tsetse fly *Glossina morsitans morsitans* for *Trypanosoma brucei brucei.* This work was carried out in collaboration with Linda De Vooght and Jan Van Den Abbeele of the Institute of Tropical Medicine in Antwerp, Belgium. The results indicate that an irradiation treatment of 110 Gy reduced the trypanosome infection rate in salivary glands and the midgut, i.e. from 14% to 6% and from 18% to 6%, respectively (Fig. 1).
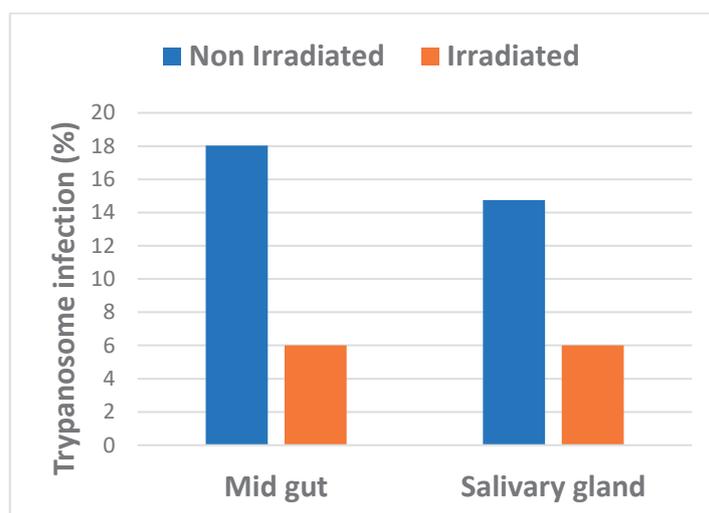

FIG. 1: *Impact of irradiation on the susceptibility of Glossina morsitans morsitans for trypanosomes (infection determined by light microscope observation of trypanosomes at 100x magnification.*

Impact of ionizing radiation on cuticular hydrocarbon profile of tsetse flies

Released sterile male tsetse flies need optimal competitiveness to compete successfully with wild males for matings with wild females. This is a fundamental requirement for the successful implementation of a SIT programme. The irradiation treatment might affect the performance of sterile males with respect to survival and might alter the male-associated microbiota. In addition, the effect of irradiation on the cuticular hydrocarbons (CHC) is poorly understood. These chemicals are ubiquitous and both structurally and functionally diverse in insects. Although the primary function of CHCs is to protect the insect from water loss, they have a multitude of functions in intra- and interspecific communication, both in a solitary as well as a social context. In particular, CHCs play an important role in mate attraction, species and sex recognition, courtship, and mate choice in many insect species. We investigated the impact of irradiation (20, 50 and 110 Gy) on *Glossina morsitans morsitans* males (22-day and 29-day old pupae and adults) and the results indicate that irradiation has no effect on the CHC profile (Fig. 2).
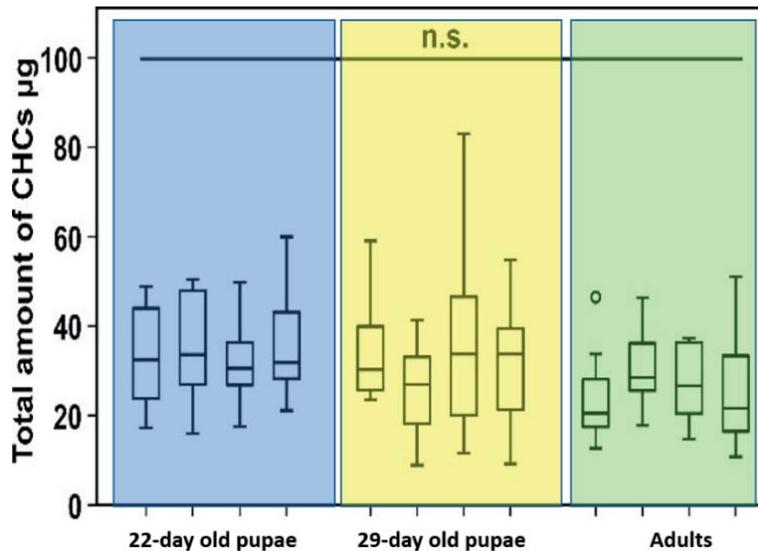
FIG. 2: *Impact of irradiation (dose of 20, 50 and 110 Gy) administered to 22 and 29-day old pupae and adult* Glossina morsitans morsitans *on the profile of the cuticular hydrocarbons. ns: no significant difference between different treatment*

Impact of ionizing radiation of tsetse fly fertility

The sterile insect technique was successfully used to eradicate a population of the tsetse fly *Glossina austeni* from Unguja Island, Zanzibar, Republic of Tanzania in 1997. Thereafter, there were attempts to implement the SIT in several countries in mainland Africa, i.e. Ethiopia and Senegal. However, due to the special biology of the tsetse, i.e. their low reproductive capacity and being hematophagous, the rearing of tsetse flies in large numbers represents a significant challenge. The establishment of large tsetse mass-rearing facilities to produce enough sterile males for the implementation of the SIT requires a capital investment. Therefore, smaller projects like the eradication of a tsetse fly population(*Glossina palpalis gambiensis*) from the Niayes around Dakar, Senegal, adopted the strategy of procuring the sterile male pupae from abroad, namely the Centre International de Recherche -Developpement sur L'Elevage en Zone Sub-humide (CIRDES) in Burkina Faso, the Slovak Academy of Sciences (SAS), Bratislava, Slovakia and the Insect Pest Control Laboratory (IPCL), Seibersdorf, Austria. As the sterile males might not be 100% sterile, some concerns were raised with respect to the potential of introducing new genetic material in the target area, in cases where the strain released does not originate from the release area

To evaluate the fate of offspring from irradiated male matings with untreated females, *G. morsitans morsitans* males were exposed as 29-day old pupae to various doses of ionizing radiation (20, 50, 70, 90, and 110 Gy). Non-irradiated male pupae served as the control. After emerged males were mated with virgin females, the mortality of the males and the productivity of the mated females were recorded. The results indicated that the survival of males was significantly reduced with irradiation doses of 50, 90 and 110 Gy in comparison with the non-irradiated control (Fig. 3). In addition, there was a significant decrease in the productivity of mated females with increasing irradiation dose of the males. To assess the productivity of the F_1 offspring, emerged F_1 males were mated with 3-day old virgin females and emerged F_1 females were mated individually with 7-day old males; the productivity and mortality of both combinations were recorded. The results indicate that productivity of females mated with F_1 males was not affected by the irradiation doses used for the parent, however the productivity of F_1 females mated with 7-day old males was significantly reduced with increasing dose used for the parents (Fig. 4).
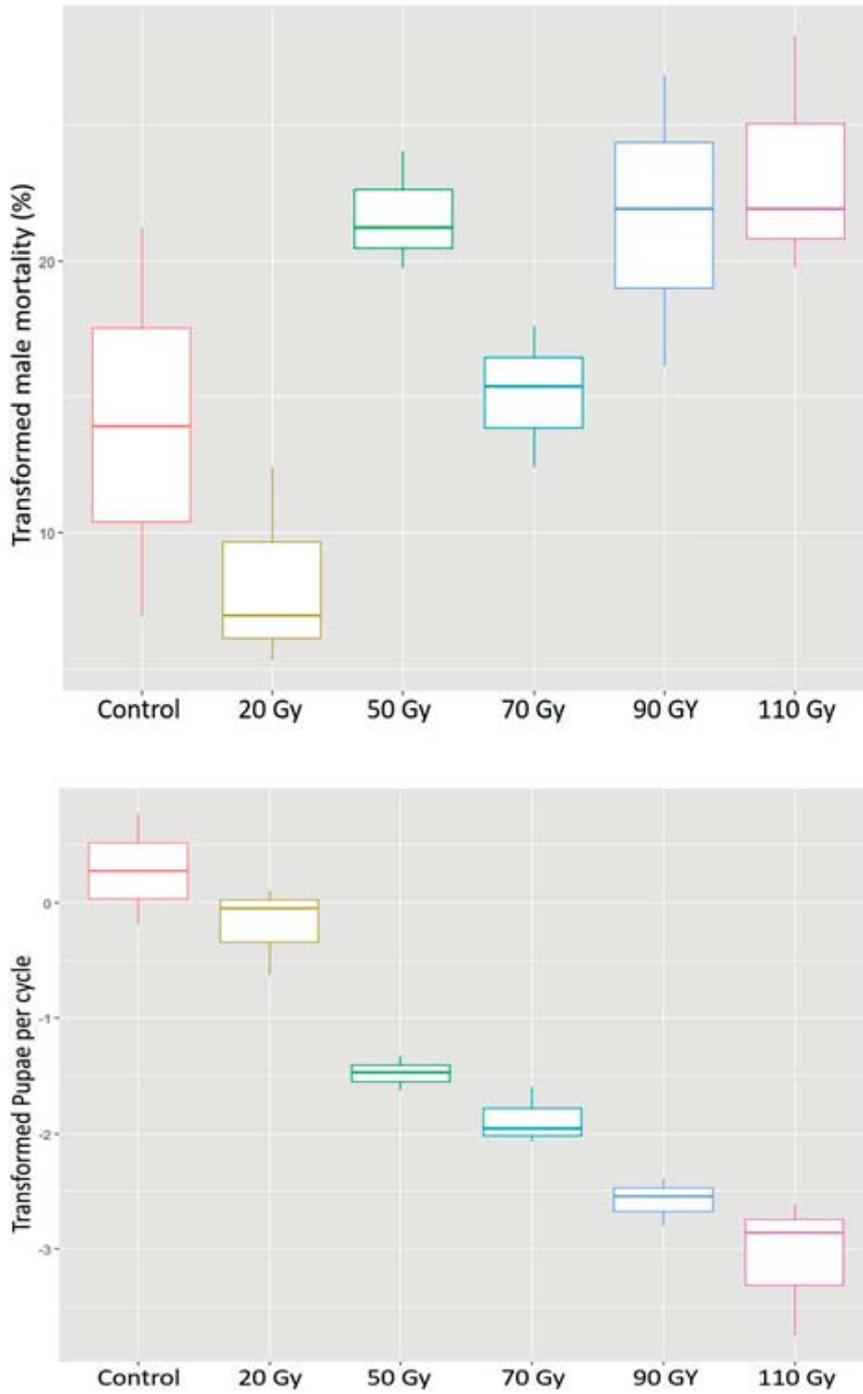
FIG. 3: *Mortality of male* Glossina morsitans morsitans *exposed to increasing radiation doses (top), and pupae production of virgin females mated with the irradiated males (bottom)*
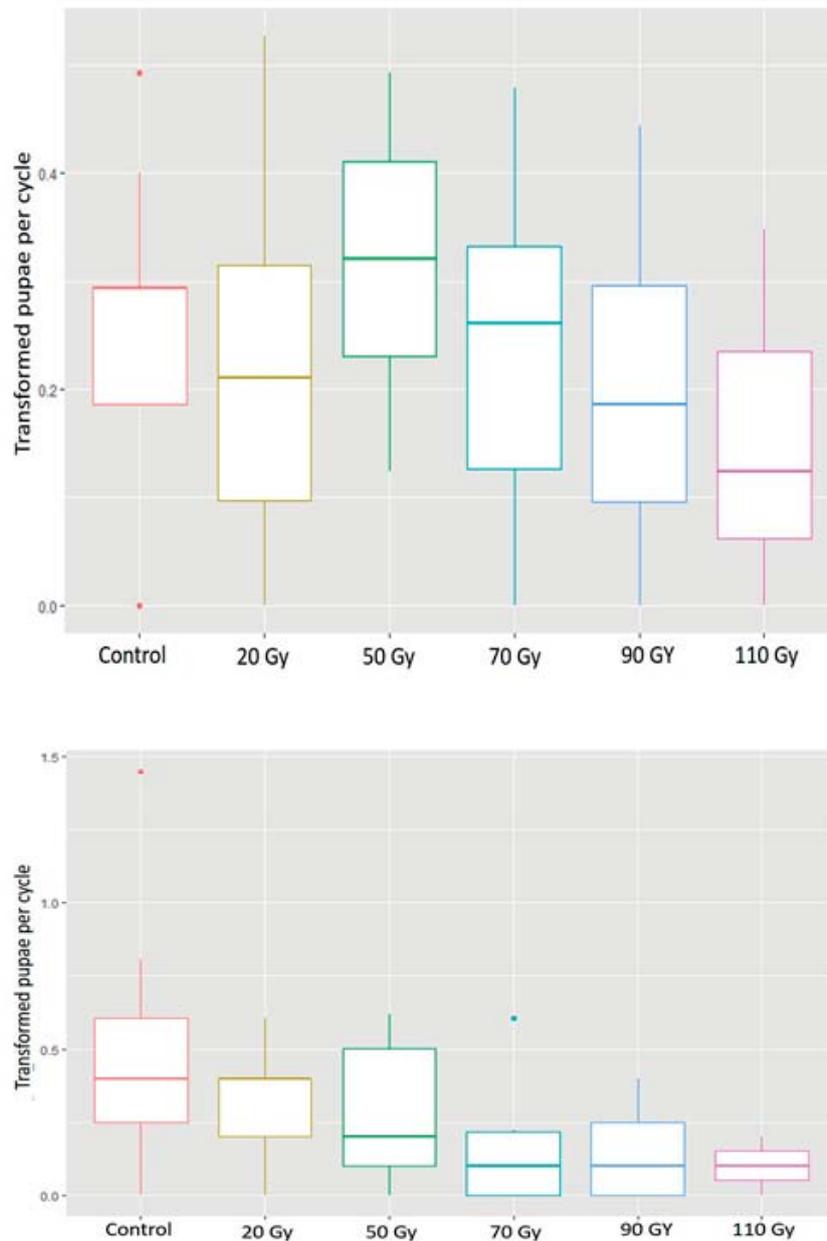
FIG. 4: *Productivity of untreated colony females mated with F$_1$ males (top), and productivity of F$_1$ females mated with 7-day-old untreated colony males(bottom).*

Near infrared pupal separation

In the sterile insect technique, it is the sterile males that have the biggest impact by inducing sterility in the wild females, as males can mate with several females. Often the presence of sterile females mixed with the sterile males reduces the efficacy of the SIT and in the case of tsetse flies, it is essential to keep all the females for the production colony due to their very slow reproduction.

Previous work has shown that near infrared (NIR) spectroscopy can distinguish male from female tsetse in the pupal stage about 5 days before emergence, potentially allowing the male pupae to be handled and sterilized separately which leaves the females available for the colony. However, this proved too unreliable to be practical and the reasons became clear when NIR macrophotography of the pupae was used. The time at which the NIR spectroscopy works coincides with the stage of

development of the pupa when the wings of the females are melanising, two days ahead of the male wings melanising. The wings are however wrapped underneath the abdomen and only visible when the pupae have the correct orientation. As the spectrometer did not control the orientation, many females with darkening wings were not identified.
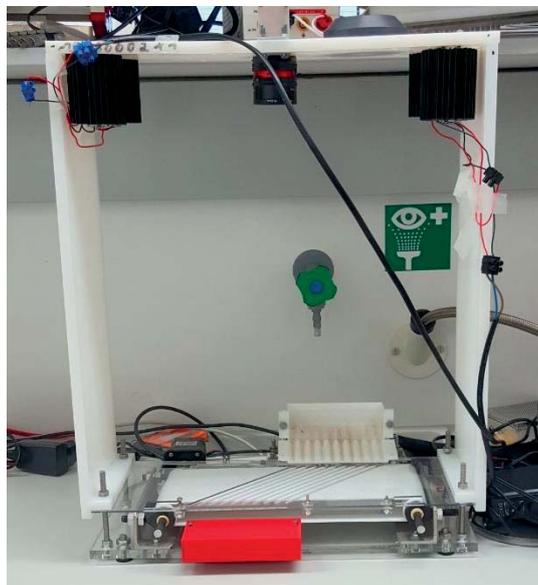
FIG. 5: Prototype of a near infrared spectroscopy system to separate male from female tsetse pupae

Therefore, systems are being developed to overcome this problem (Fig. 5). The main system currently feeds the pupae individually onto a moving belt that causes the pupa to rotate. A high-speed NIR video camera captures images of the pupa as it rotates, and the orientation of the pupa can be determined from the position of the breathing lobes at the end of the pupa. The images from the appropriate orientation (ventral side up) can then be analysed to determine the sex.

It is planned to develop an operational system from the current prototype by the end of 2019.

X-ray systems to replace isotopic irradiators.

The sterile insect technique relies upon having an efficient and safe method for sterilizing the insects to be released. For many years this has been accomplished using isotopic irradiators, using either 60-cobalt or 137-caesium as the active isotope in self-shielded and panoramic irradiators. Although these irradiators have proved to be very safe, the possibility for accidental or deliberate misuse remains and, as a matter of policy, the IPCL has been searching for non-isotopic alternatives. The alternatives, however, must be affordable and have sufficient capacity to meet the demand.

Machine sources of radiation fall into two basic categories: low energy X-rays generated in orthovoltage tubes (like medical X-rays) and high energy electron accelerators, either utilizing the electron beam directly or converting it to X-rays with a metal foil target. Accelerators have the potential to be efficient and have high capacity, but they are also currently too expensive to be used in SIT programmes. Discussions are underway with accelerator manufacturers to design smaller, cheaper units for the future.

Low voltage X-ray (150 – 250 keV) units are available from many manufacturers, but most suffer from low power and poor dose distribution due to the beam configuration. In these units the X-ray tube is placed at the top of a shielded cabinet facing downwards onto a table on which the sample is placed. This gives a reasonable dose distribution across the beam width but poor uniformity with depth into the sample, limiting the usable depth for SIT to less than 10 mm to achieve a dose uniformity ratio of less than 1·3. Dose uniformity can be improved by turning the sample over or rotating it continuously in the beam, but this is not practical in this configuration.

FIG. 6: Nuctech X-Lab prototype twin horizontal beam X-ray irradiator © A.G. Parker

In conjunction with an X-ray system manufacturer, we have been working to design a more efficient system. Instead of having the tube shine vertically downwards, the beam is oriented horizontally so the sample can be simply rotated about a vertical axis (Fig. 6). In addition, the load is divided into two canisters stacked on top of each other, so that the two can be swapped half way through irradiation to smooth out the vertical dose variation. Together these changes can easily achieve the desired dose uniformity ratio of less than 1·3. Adding a second X-ray tube opposed to the first increases the available power, giving a dose rate of about 8 Gy.min-1 to a volume of almost 3 litres. Such a system has a capacity to irradiate 150,000 fruit fly or tsetse pupae in about 12 minutes to give an overall capacity of 24 million pupae per week.

Plant Pests

Phytosanitary treatments under the FAO/IAEA/USDA agreement

Phytosanitary irradiation and cold treatment experiments were continued under the FAO/IAEA/USDA agreement on "Harmonization of phytosanitary treatments for exotic fruit flies" (Fig. 7). This agreement aims to develop broadly applicable phytosanitary treatments against economically important tephritid species. The phytosanitary research carried out at the IPCL can be used as a guideline to develop treatment schedules in FAO and IAEA Member States. After publication, the results from our phytosanitary research can also be used to propose new phytosanitary treatments and amendments to the international standards recommended by the International Plant Protection Convention (IPPC). Ultimately, the trade among Member States might be facilitated through the application of the phytosanitary treatments proposed by our group, thus improving quarantine security and food safety worldwide.

FIG. 7: Ms Olga Barrera (Colombia), Mr Luis Caravantes (Mexico), and Mr Alexandre Araujo (Brazil) dissecting irradiated mangoes for phytosanitary research at the IPCL

Prior studies focussing on the definition of target doses against fruit flies have significantly contributed to advancing the use of phytosanitary irradiation in Member States. However, more research is needed to determine whether low-oxygen atmosphere conditioning, a treatment widely applied to preserve commodity quality, can increase the radiotolerance of fruit fly species and reduce the efficacy of irradiation treatments. Preliminary results from our group suggest that hypoxic and severe-hypoxic conditioning before and during irradiation can increase the survival of *Anastrepha fraterculus* (Brazilian-1), *Anastrepha ludens*, *Bactrocera dorsalis*, and *Ceratitis capitata* only at low doses of gamma radiation. At high doses of irradiation, low-oxygen conditioning treatments did not increase the survival rates of any fruit fly species evaluated. Once published, these results can contribute to the revision of restrictions applied by regulatory agencies to phytosanitary irradiation for commodities conditioned under low oxygen levels.

Comparative research on cold treatment against four populations of the *Anastrepha fraterculus* complex using naturally infested nectarines has been completed. No major differences in cold tolerance was found among the Andean (Colombia: Ibagué), Brazilian-1 (Argentina: Castelar and Tucuman), Ecuadorian (Peru: Cusco), and Peruvian (Peru: La Molina) morphotypes. This research will help guide regulatory decisions that may result from the description of new species out of that complex and help avoid trade barriers.

Drosophila suzukii

The rapid dispersal of *Drosophila suzukii* and its subsequent economic losses encouraged the development of different approaches towards its management. The SIT can potentially be integrated in area-wide integrated pest management (AW-IPM) approaches to manage this pest under confined environment systems such as greenhouses. Staff of the IPCL have been working on the development of the SIT package for *D. suzukii* including determination of the optimum sterilization dose, development of effective mass-rearing procedures (Fig. 8) and quality control protocols, as well as the assessment of their mating behaviour.

Cold treatment of pupae during irradiation

Mr Thomas Enriquez, a PhD student from the University of Rennes in France, was hosted by the IPCL to assess the effect of cold treatments on the quality of irradiated *Drosophila suzukii*. There are several steps in the process of mass-producing insects towards the actual field release that may compromise the quality of the insect and therefore the success of the SIT operation. Irradiation to sterilize the insects is an event that may induce somatic damage that is partially due to oxidative stress. The antioxidant capacity of insects might be increased by conditioning them before or during irradiation using e.g. a low oxygen atmosphere (hypoxia). Similarly, when insects are exposed to cold temperatures, they produce antioxidants and heat shock proteins to better tolerate the stress. Consequently, it was hypothesized that cold exposure of *D. suzukii* pupae before irradiation may increase the production of antioxidants, offering better protection from the deleterious effects of irradiation. A large array of cold treatments was tested, but none of them significantly increased insect quality after irradiation.


FIG. 8: Ms Fabiana Sassu (PhD student) from Italy with an adult holding cage for Drosophila suzukii

Shipment of sterile insects after irradiation is another key step of a SIT programme. Generally, insects are shipped as pupae, therefore shipping conditions need to be adapted to prevent adult emergence during transport and before release in the target area. Pupae are often shipped under cold or hypoxia conditions, however the effects of long-term cold exposures on irradiated *D. suzukii* pupae are presently unknown. Furthermore, hypoxia is stressful in itself and may induce damage if the exposure is too long. Therefore, the second aim of Mr Enriquez's experiment was to test the effect of several shipping conditions (hypoxia alone, cold alone (approximatively 0°C) or a combination of both treatments) on the quality of irradiated *D. suzukii* pupae. The shipping conditions were simulated at the IPCL and treatments lasted for 24 or 48 h. None of the treatments had a negative effect on emergence rate of pupae, but after 48 h of hypoxia alone, emerged flies were not capable of flying. When hypoxia was combined with a cold treatment, the flying rate of the treated flies was similar to that of untreated flies. In conclusion, we showed that the use of cold treatment in combination with hypoxia seemed to be a promising method to avoid emergence of flies while preserving their quality. Supplementary experiments are planned to investigate the physiological basis of these phenotypical data.

The IPCL has been collaborating with the 'Fondazione E. Mach - Dep. Sustainable Agroecosystems and Bioresources, Agricultural Entomology Unit, San Michele, All. Adige, Italy' on a project to assess some aspects of the mating behaviour of *D. suzukii*. The focus of this project is on sperm competition and sperm displacement between sperm from sterile or fertile males. As part of this experiment, irradiated and fertile pupae have been shipped weekly from the IPCL to San Michele, Italy. The pupae were shipped in styrofoam boxes containing ice packs to maintain a constant temperature. To assess the effect of shipping duration and packing conditions on the pupae, quality control tests were carried out at the IPCL (before shipment) and at San Michele, Italy, upon receipt of the pupae.

Sterile male sexual competitiveness

Experiments were designed in small laboratory cages to determine the ability of sterile males to compete with fertile males for mating with fertile females from the same laboratory population. The tests were carried out under controlled laboratory conditions to observe mating behaviour and interactions during the time of sexual activity. Sterile and fertile insects were released in the laboratory cages and the number of successful matings achieved by the fertile and sterile males with fertile females was recorded.

The pupae were irradiated with 220 Gy in a ^{60}Co source under normal atmosphere conditions (normoxia) or under hypoxia conditions. The aim of the tests was to assess whether hypoxia conditions could mitigate some of the negative effects of the irradiation and potentially improve their mating performance as compared with the males treated under normoxia conditions.

Preliminary results indicate that irradiated males were less successful in mating with the females as compared with fertile males, but males irradiated under hypoxia conditions achieved more matings than sterile males irradiated under normoxia conditions.

The South American fruit fly

As reported previously, the Plant Pest group has been working on the development of a genetic sexing strain (GSS) of the South American fruit fly *A. fraterculus* that is based on a pupal colour dimorphism (brown-black) resulting from a reciprocal translocation between the Y chromosome and the autosome carrying the wild type locus of the black pupae (*bp*) gene. After a preliminary evaluation that assessed the genetic stability, the production and quality control profile of the strain, the line "IPCL – 89" was selected for characterization under mass-rearing conditions. A cytogenetic analysis was carried out by Antigone Zacharapoulos of the University of Patras, Greece, to characterize the chromosome involved in the reciprocal translocation and break points.

Human Disease Vectors

The efficiency of a new automated mosquito larval counter

To achieve consistent and standardized rearing of mosquitoes at immature stages, it is important to control the initial number of larvae present in each larval tray. In addition, maintaining an optimal and synchronized development rate of larvae is essential to maximize the pupal production and optimize male sorting in a mass-rearing setting. Manual counting is labour-intensive, time-consuming and error-prone. We evaluated the use of a customized automated counter for the quantification of mosquito larvae. The present prototype of the mosquito larval counter uses a single counting channel consisting of three parts: a larvae dispenser, an electronic counting unit and computer control software. The prototype was very efficient to count mosquito larvae and provided repeatable and reproducible results without impacting mosquito quality. The error range was acceptable for our purposes as the time burden has been significantly reduced. Refinement (with a bigger larvae input container and a configuration with multiple channels) is ongoing, aiming to reduce the number of operations and ultimately speed up the process for potential use in mass-rearing settings, which requires 18,000 first instar larvae per rearing tray. This technology could also be used in the future to evaluate larval density in the tray before pupal collection as a quality control tool to estimate mortality.
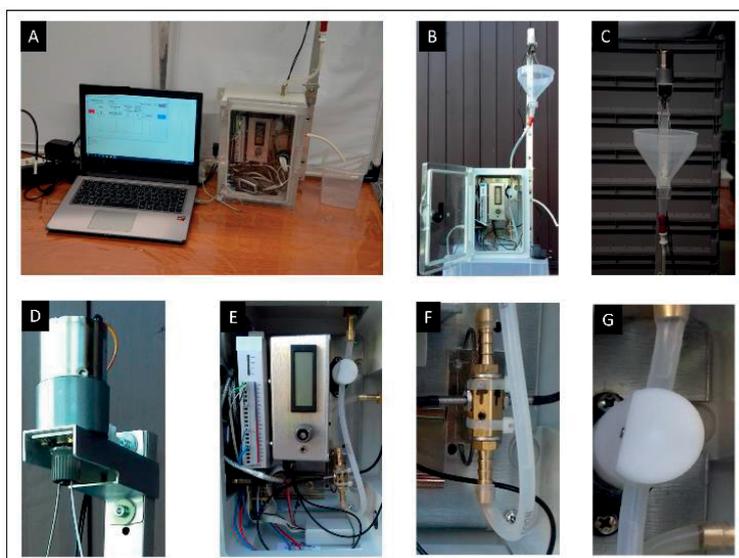
FIG. 9: The automated mosquito larval counter. General view of the larval counter (A); general display of the electronic counting unit and the input larvae container (B); funnel as input larvae container (C); stirrer unit (D); electronic counting unit (E); optical sensor head (F); pinch valve (G).

Developing cost-effective mosquito larval diets to reduce rearing costs

Larval diet is one of the most critical and costly components in the application of mosquito SIT. As part of the development of the mosquito SIT package at the IPCL, a standard artificial larval diet consisting of powdered tuna meal, bovine liver powder and brewer's yeast was developed earlier and is currently being used efficiently in the IPCL and in other laboratories. Although this reference diet adequately supplies the necessary components for larval growth including sugars, fatty acids, proteins and vitamins, one of the ingredients (bovine liver powder) is expensive and not widely available in some Member States. Availability and affordability of diet components are important for developing an artificial diet. Hence, alternative cost-effective and readily available diet ingredients that can be procured in large quantities for mass-rearing of *Aedes sp*. are urgently needed. Insects such as *Tenebrio molitor* (yellow mealworm), *Musca domestica* (house fly) and *Hermetia illucens* (black soldier fly) were tested for their potential use as proteins to feed mosquito larvae. Results were promising

regarding mosquito development and quality parameters. Importantly, black soldier fly powder allowed a cost reduction of up to 80% in comparison to the current larval diet.

FIG. 10: New mosquito larval diet component. From the left to the right, adult, mature larvae and pupae and grounded powder of Black Soldier Fly, used to reduce the cost of mosquito larval diet.

Optimizing blood feeding for mass-rearing of Aedes aegypti

The sterile insect technique relies on the release of large numbers of sterile male insects in the target area and the feeding of blood for females is required for egg production. In the development of a system for mass-rearing insects, cost efficiency is of utmost importance. The availability of blood collected from slaughterhouses and the management of the blood stock are also critical in a mass-rearing facility. Although artificial feeding using sausage casings has shown to be more efficient than live animals, reducing blood quantity per sausage and exploring different ways to stock blood such as freezing or irradiation in the case of unfrozen blood still requires further research.

FIG. 11: Sausage heating device developed at the IPCL to reduce the amount of blood necessary to feed a mass-rearing cage.

Different types (pig, bovine) and quality (fresh, frozen, irradiated) of blood meals were tested in terms of female fecundity, fertility and adult survival. A sausage heating device was developed and the workload and blood quantity assessed. The device is made of aluminium and can be plugged into a Hemotek heating device to maintain constant blood temperature. We confirmed that female *Aedes aegypti* had a better fecundity when fed with fresh pig blood as compared with stored pig blood. We achieved up to a 2/3 reduction in the quantity of blood necessary to feed 12,000 females using sausage casings with the heating device as compared with the older system that relied on heating the sausages in a water bath.

The use of the sausage heating device would reduce the quantity of blood needed and thus the cost in a rearing facility. However, more work is required to adapt mosquito colonies to stored blood without compromising egg production and survival while reducing cost.

Identifying factors affecting the radiosensitivity of mosquito pupae

Standardizing methods to sexually sterilize mosquitoes using ionizing radiation is essential to obtain reliable and reproducible results in the frame of the SIT. Numerous factors affect the physical and biological effects during irradiation procedures. Some have been identified and characterized while others still need careful assessment.

It has been shown that older pupae (or life stages) are more resistant to radiation than younger ones. To assess the extent of the relationship between age and effect, five groups of *Aedes aegypti* pupae of increasing age were irradiated simultaneously at a fixed dose and level of sterility, and their longevity was assessed. There was a strong negative correlation between pupal age and radiosensitivity even when age differences were small. A 6% difference in residual fertility was observed between the youngest age groups (10-24 hours) and oldest age groups (45-50 hrs) when exposed to 40 Gy.

Longevity of the adult sterile males was also affected by the age at which they were irradiated. A dose of 40 Gy administered to adult males younger than 24 hrs reduced their mean longevity by 40% compared with untreated controls, while those irradiated when older than 42 hrs survived longer (25%) than the untreated controls.

The size of the *Aedes aegypti* pupae did not affect levels of sterility. Small pupae (<0.900mm) and large pupae (>1.100mm) of the same age and cohort were irradiated simultaneously at a diagnostic dose of 30 Gy. Colony females that had mated with males emerging from these small and larger pupae had a mean induced sterility of 76.5% and 75.9%, respectively.

Handling and sample preparations for pupae irradiation can significantly alter the biological responses, especially when the atmospheric conditions change. Submerging pupae in water during irradiation induced changes in the level of dissolved oxygen as pupae continue to absorb oxygen under water. Lowering oxygen levels creates a hypoxic environment that changes cellular responses and provides radioprotective effects. Pupae irradiated in such an environment suffer less damage and therefore have a higher residual fertility than pupae irradiated in air. Experiments comparing induced sterility following irradiation of pupae in water and in air confirmed the reduced radiosensitivity in 3 mosquito species – *Ae. aegypti*, *Ae. albopictus*, and *An. arabiensis*.

Several additional factors which may impact dose-response are under investigation and will provide important information for the development of standardized protocols for irradiating mosquitoes in the frame of the SIT.

Use of stable isotopes in mosquito mating studies

Stable isotopes can be useful tools to study the mating behaviour and the fate of sperm in mosquitoes. Different groups of males, i.e. sterile versus fertile males, or males of different strains, can be marked with isotopes such as ^{13}C and ^{15}N, and after the mating, the presence (or absence) of these isotopes can be detected in female spermathecae. Previous studies indicated that virgin females can accept sperm from two males if they mate within 20 minutes of each other, and further experiments were designed to assess the potential mixing of sterile and fertile sperm in the spermathecae, and whether sperm selection (for the "better" fertile sperm) occurs in double-mated females. Preliminary data indicate that sperm distribution and use is random, and there is no evidence of sperm selection or sperm precedence. Essentially, the first male that finds and mates the female will father future offspring. If both sterile and fertile males mate with a female within a 20-minute window, both fertile and sterile sperm will be used and will result in the intermediate sterility.

This information provides important insight in *Aedes* mosquito mating behaviour which will assist in the improvement of sterile male release strategies.

Novel quality control tool adapted to Anopheles arabiensis

In NL 91 we reported that a simple flight ability test was developed that accurately predicted the quality of male *Aedes aegypti* and *Ae. albopictus* and was validated through a series of stress tests. Similarly, there is currently no standardized method of assessing the quality of male *Anopheles arabiensis* mosquitoes. Therefore, the flight ability device developed for *Aedes* was used in a series of preliminary experiments for *An. arabiensis,* revealing that the diameter of the individual flight tubes was too narrow for the slightly larger body size of male *An. arabiensis*. We therefore amended the device using slightly wider flight tubes (1 cm of internal diameter). Initial testing was successful and subsequently a series of 10 new flight ability devices were produced for *An. arabiensis*.

FIG. 12: A prototype flight test device for Anopheles arabiensis

Experiments are currently underway to expose *An. arabiensis* males to various stress treatments including irradiation, chilling and compaction, similar to those used during the tests done with *Aedes* species earlier last year (2018). Survival and insemination data will be used as reference methods and compared to the flight ability results. We expect this tool to be validated soon and thus provide a useful tool to measure quality in another important human disease vectors.

Genetics and Molecular Biology

Towards the development of mosquito genetic sexing strains: evaluation of mutations in Aedes aegypti

Morphological mutations are a necessity to construct a genetic sexing strain (GSS) using classical genetic approaches. The availability of a good GSS is of great importance for mosquito SIT applications. In the case of *Aedes aegypti*, the M factor is known to be in a small, non-recombining area on chromosome I and its presence is sufficient to lead to male development. Therefore, morphological mutations that are in chromosome I and are tightly genetically linked with the M locus are of special interest. The properties and the possible incorporation in a strategy to develop a GSS of two such morphological mutations are being evaluated. Our results so far show that these mutations are recessive, with full penetrance. The recombination frequencies between these mutations and the M locus are being evaluated. The current available data indicate that the recombination frequencies show transgenerational stability and do not seem to be influenced by the age of the adults, as evident from recombination data of consecutive gonotrophic cycles. On the other hand, these recombination

frequencies need to be reduced or even better, eliminated, to be effectively incorporated in a genetic sexing strategy.

A. Genetic sexing strain without inversion
(recombination frequency ranging between 2-3%)

Genetic distance (2-3%)

M → x⁺

m   x

B. Genetic sexing strain with inversion
(recombination frequency reduced to <0.3%)

Genetic distance (<0.3%)

M → x⁺

m   x
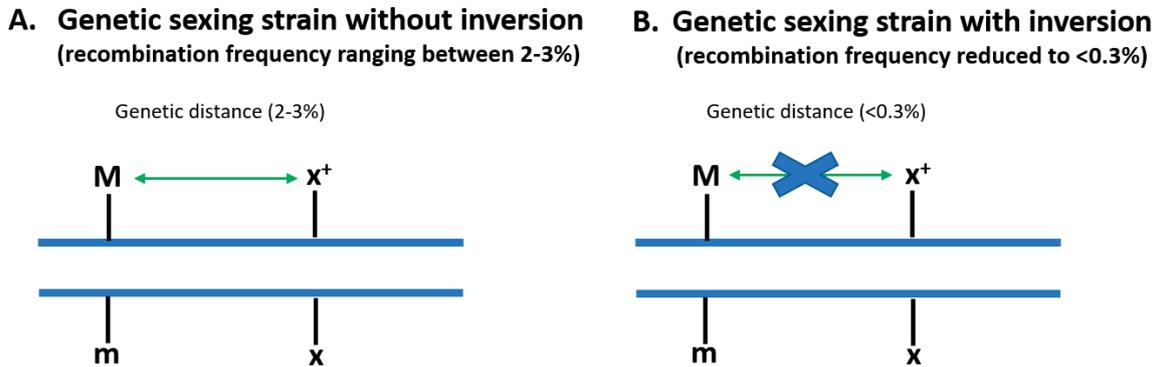
FIG. 13: Increased genetic stability of the Aedes aegypti genetic sexing strains through the introduction of an inversion that reduces recombination between the M locus and the wild type marker (x+).

### Towards the development of mosquito genetic sexing strains: evaluation of chromosomal inversions in Aedes aegypti

High recombination frequencies observed in *Aedes aegypti* males (comparable with recombination frequencies of females) can significantly affect the genetic stability of genetic sexing strains, including those we have developed using morphological markers linked to the M locus. To enhance the genetic stability of the constructed genetic sexing strains, we have been searching for recombination suppressors such as chromosomal inversions. Inversions are known to reduce recombination within their range and this effect can be extended to neighbouring genomic areas. Therefore, selecting the appropriate inversions could reduce or even eliminate recombination between the two loci of interest (M locus and selectable marker) and provide a GSS of higher genetic stability (2nd generation GSS).

We applied two different strategies to isolate such chromosomal inversions. In the first approach, knowing that inversions can be present in natural populations, we screened laboratory colonies available in the IPCL that were derived from different geographic areas. In the second approach, we used irradiation to induce chromosomal rearrangements to identify inversions with the desirable recombination-reducing characteristics. In both approaches, screening was performed based on reduction of recombination among the loci of interest (sex determining region and selectable morphological markers). One chromosomal inversion with the desirable characteristics was isolated which was shown to reduce about 10 times the recombination frequency observed between the M locus and the morphological marker (Fig. 13).

Genetic changes of Ceratitis capitata during laboratory adaptation.

The successful implementation of the sterile insect technique requires the production of large numbers of high-quality sterile insects that will be released in the field. Mass-rearing is a crucial step for SIT because it allows an industrialized sterile insect production. Mass-rearing of insects often produces individuals adapted to the mass-rearing environment and that are different from their wild counterparts. This phenomenon known as "laboratory domestication" may alter the population at the genetic level due to changes derived from genetic drift and selective pressure of the artificial environment. Various life-history traits of the population might change including reduced developmental time, earlier reproduction, reduced longevity and dispersal ability, thus negatively affecting the population's quality and performance. It is therefore fundamental to follow these laboratory adaptation processes and link the strain mating efficiency and "wild" character with genetic markers. Microsatellite markers are highly polymorphic and abundant in the genome, and therefore they constitute a powerful genetic and molecular tool. Several microsatellite markers have previously

been developed for population genetic studies and diagnostics of the Mediterranean fruit fly. Wild Mediterranean fruit flies were collected in Greece by Prof. Nikos Papadopoulos, University of Thessaly from infested bitter oranges and were introduced in the IPCL for genetic analysis. The flies that emerged from these fruits were divided in two sub-populations and each of them was reared either in banana or on the standard carrot diet. Both populations were adapted and monitored in the laboratory for 10 generations. We selected eight markers that are highly polymorphic and can be combined in a set of 4 fluorescent dyes, without potential overlapping. Medflymic43, Medflymic30, Ccmic32, and Ccmic6 were combined and constituted the first set of our analysis, and Medflymic74, Medflymic78, Medflymic78, and Ccmic9 the second one. We used generations F_0, F_1, F_2, and F_3 from the population reared in banana, as well as some F_0 samples originated from another host (figs) to standardize our process. Polymorphism indices of the markers and a preliminary population genetic analysis were performed with Genalex - Genetic Analysis in Excel 6.503. Fig. 14 shows the allele frequencies of the marker Medflymic43 for F0, F1 and F2. Future analysis of all the markers will show whether the initial genetic diversity of the wild population changes during the domestication process.
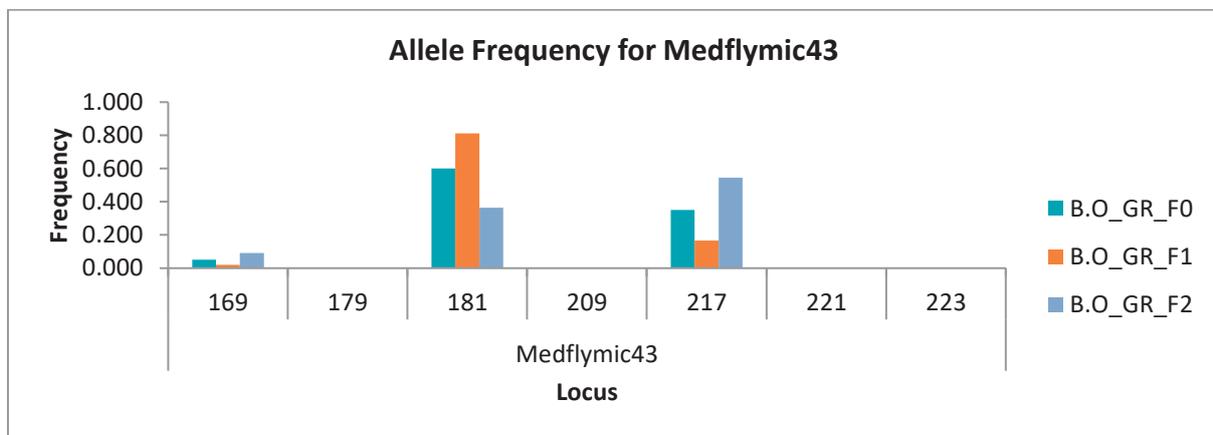
FIG. 14: *Allele frequency of Medflymic43 of the medfly population reared in banana*

Genetic changes of Drosophila suzukii *during laboratory adaptation.*

As mentioned above for the Mediterranean fruit fly, it is important to follow the laboratory adaptation processes and link the mating efficiency and "wild" character of the *Drosophila suzukii* strain, which is to be used for SIT applications, with genetic markers. Polymorphic microsatellite markers have also been developed for *D. suzukii* and they represent a promising molecular tool for population genetic studies on this pest. Prof. Hervé Colinet, University of Rennes 1, collected a founder wild population of *D. suzukii* from infested raspberries in the area of Thorigné-Fouillard in France. The collection took place in mid-September 2017 and 850 infested raspberries were brought in the laboratory and placed individually in plastic vials. The vials were kept at 20°C until the emergence of the flies. When flies emerged, one male and one female were taken from each infested fruit and placed in bottles with standard artificial diet for ovipositing. The flies from this colony were domesticated in the Insect Pest Control Laboratory using an artificial carrot diet and a rearing temperature of 23°C.

We used 4 microsatellite markers in 32 *D. suzukii* individuals from each of the following generations: F_0, F_1 and F_{10} to examine changes regarding the domestication process. DS15, DS26, DS32 and DS33 microsatellite markers were labelled with different fluorescent dyes and used for the preliminary genetic analysis. Fig. 15 shows the allele frequencies of the marker DS26 for F_1 and F_{10}. Future analysis of all the markers will show whether the initial genetic diversity of the wild population changed during the domestication process. We plan to use 12 more microsatellite markers to assess the changes in the genetic diversity and depending on the results we might include more generations.
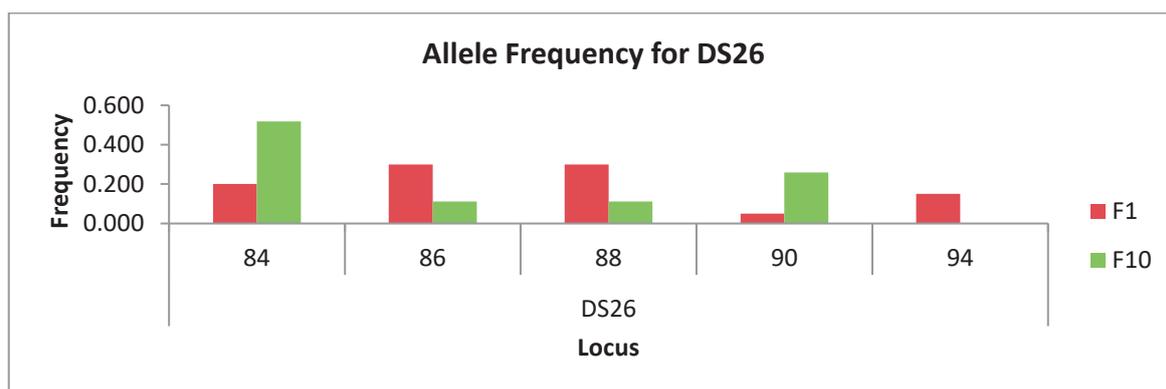
FIG. 15: *Allele frequency of DS26 of the Drosophila suzukii population reared in carrot diet*

CAPACITY BUILDING AND SERVICES

In 2018, the IPCL hosted 14 cost-free experts (CFE) and 9 consultants (C) (of which 5 were PhD students), 7 interns, 8 fellows (F) and 3 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
KOSKINOTI, Panagiota	Greece	CFE	11 mth	Olive fruit fly molecular biology and symbiosis work
RIVERA, Camilo	Guatemala	Intern	6 mth	Phytosanitary treatments
GUILLEN RIVERA, Martha	Mexico	Intern	8 mth	Characterising GSS of fruit flies
NOLASCO GOMEZ, Abner	Mexico	Intern	12 mth	Characterising GSS of fruit flies
ALVAREZ, Mario	Mexico	Intern		Characterising GSS of fruit flies
MARTINEZ BARRERA, Olga	Colombia	Intern	8 mth	Phytosanitary treatments
AMORIM DA SILVA CARDOSO, Amanda	Brazil	Intern	12 mth	Phytosanitary treatments
VARGAS HURTADO, Nick	Mexico	Intern	12 mth	Phytosanitary treatments
HALLMAN, Guy	USA	C	2 weeks	Phytosanitary treatments
ELGHADI, Elsam	Libya	F	2 weeks	Fruit fly rearing

Name	Country	Status	Duration	Topic
DEMIRBAS, Gueler	Turkey	C (PhD)	3 mth	Tsetse symbionts and pathogens
MEKI, Irene	Kenya	C (PhD)	3 mth	Management strategies for tsetse pathogens
MIRIERI, Caroline	Kenya	C (PhD)	8 mth	Impact of stress factor on tsetse flies
DIENG, Mouhamadou	Senegal	C (PhD)	5.5 mth	Tsetse symbionts and refractoriness to trypanosome infection
REZAPANAH, Mohammadreza	Iran	CFE	4 mth	Tsetse pathogens
SASSU, Fabiana	Italy	CFE (PhD)	12 mth	Radiation biology and rearing of *Drosophila suzukii*
NIKOLOULI, Katerina	Greece	CFE (PhD)	12 mth	*Wolbachia* in *Drosophila suzukii*
WALLNER, Thomas	Austria	C	3 mth	Rearing of mosquitoes
CARAVANTES, Silvana	Guatemala	CFE	9 mth	Rearing fruit flies
CULBERT, Nicole	UK	C (PhD)	11 mth	Mosquito handling techniques
DIAS DE CASTRO, Vanessa	Brazil	C	12 mth	Phytosanitary treatments
RAMIREZ OSORIO, Adriana	Mexico	CFE	1 mth	Mosquito genetics
ZACHAROPOULOU, Antigone	Greece	CFE	1 wk	Fruit fly cytogenetics
DROSOPHOULOU, Eleni	Greece	CFE	1 wk	Fruit fly cytogenetics
GOUVI, Georgia	Greece	CFE	1 wk	Fruit fly cytogenetics
MARKOU, Angeliki	Greece	CFE	1 wk	Fruit fly cytogenetics
PRIMO, Pasquale	Italy	CFE	2 mth	Fruit fly genetics and molecular biology

Name	Country	Status	Duration	Topic
HERRANZ, Gustavo Salvador	Spain	C	1 wk	Mosquito sex separation
GOMEZ PACHECO, Maylen	Brazil	F	1 mth	Mosquito genetics
BOND, Juan Compean	Mexico	F	1 mth	Mosquito genetics
KORTI, Mohammed	Sudan	F	4 mth	Marking & release of sterile male *Anopheles*
MAZZEI ANDRADE, Mauro	Argentina	F	3.5 mth	Compatibility studies of *Aedes* mosquitoes
MEAD, Lucas	Jamaica	F	6 mth	Compatibility studies of *Aedes* mosquitoes
GARCIA ALBA, Marianela	Argentina	F	4 mth	Compatibility studies of *Aedes* mosquitoes
DIEUDONNE SOMA, Diloma	Burkina Faso	F	3 mth	Mass-rearing of mosquitoes
RESILVA, Sotero	Philippines	SV	1 wk	Mass-rearing of mosquitoes
OBRA, Glenda	Philippines	SV	1 wk	Mass-rearing of mosquitoes
PAGABELEGEUM, Soumaila	Burkina Faso	SV	1 wk	Planning field operations for tsetse SIT in Burkina Faso
GHOUATI, Kamil	France	CFE	1 wk	Public acceptance of mosquito SIT
PLEYDELL, David	France	CFE	1 wk	Modelling of mosquito SIT
BRUNO, Jimmy	France	CFE	1 wk	Automatic release system for tsetse
RABITSCH, Ingrid	Austria	CFE	1 wk	Mass-rearing of mosquitoes

In 2018, the Genetics and Molecular Biology (GMB) group maintained 13 species of fruit flies (160 strains/colonies/populations) and 3 species of mosquitoes (69 strains/colonies/populations in total).

The Plant Pests (PP) group maintained 14 species of fruit flies (65 strains/colonies/populations), the Livestock Pests (LP) group maintained 7 tsetse species (11 strains) and the Human Disease Vectors (HDV) group maintained 3 mosquito species (15 strains)

65

The GMB/PP groups carried out 64 fruit fly shipments to 18 institutions (Senegal, Greece, Germany, Italy, Bangladesh, Uruguay, Czech Republic, Pakistan, Brazil, Sweden, UK, Spain, Kenya, Mauritius, Mexico and France), and 2 shipments of preserved fruit flies to 2 institutions (Italy and Belgium).

The LP group carried out 148 tsetse shipments of 270,407 pupae (239,744 *G. palpalis gambiensis* to Senegal) to 9 institutions in 8 countries (Senegal, France, Uganda, USA, Belgium, Zimbabwe, South Africa and Netherlands).

The HDV group carried out 14 mosquito shipments to 8 institutions (Spain, Sweden, Brazil, Germany, Mexico, Austria, France and Switzerland).

In 2018, the IPCL received 672 visitors from 88 countries.

PUBLICATIONS

ABD-ALLA, A.M.M., G. TSIAMIS and D.G. BOUCIAS, (2018). Special issue on enhancing vector refractoriness to trypanosome infection-foreword. BMC Microbiolology 18(Suppl 1):141.

AHMAD, S., C. CÁCERES, U. STO TOMAS, T. DAMMALAGE, K. GEMBINSKY, M.J.B. VREYSEN et al. (2018). One for all: Mating compatibility among various populations of olive fruit fly (Diptera: Tephritidae) for application of the sterile insect technique. PLoS ONE 13(11):e0206739.

AHMADI, M., B. SALEHI, A.M.M. ABD-ALLA, M. BABAIE (2018). Feasibility of using the radiation-based sterile insect technique (SIT) to control the olive fruit fly, *Bactrocera oleae* Gmelin (Diptera: Tephritidae) in Iran. Applied Radiation and Isotopes 139:279-284.

AUGUSTINOS, A., I.K. MEKI, A.G. PARKER, A.M.M. ABD-ALLA, K. BOURTZIS et al. (2018). Nuclear and *Wolbachia*-based multimarker approach for the rapid and accurate identification of tsetse species. BMC Microbiology 18(Suppl 1):147.

BOURTZIS, K. and Z.J. TU (2018). Joint FAO/IAEA Coordinated Research Project on "Exploring genetic, molecular, mechanical and behavioural methods of sex separation in mosquitoes" – an introduction. Parasites & Vectors 11 (Suppl 2):653.

BOUYER, J. and M.J.B. VREYSEN (2018). Vectors: tsetse flies. In: Infectious Diseases of Livestock, J.A.W. Coetzer, G.R. Thomson, N.J. Maclachlan, M.-L. Penrith and A. Michel (Eds.). Anipedia (www.anipedia.org).

BOUYER, J. and R. LANCELOT (2018). Using genetic data to improve species distribution models. Infection, Genetics and Evolution 63:292-294.

BOUYER, J. and E. MAROIS (2018). Genetic Control of Vectors. In: C. Garros, J. Bouyer, W. Takken and R. Smallegange (Eds.). Pests and vector-borne diseases in the livestock industry: Ecology and control of vector-borne diseases. Wageningen Academic Publishers, The Netherlands. p. 435-451.

BIMBILE, SOMDA N.S., H. MAIGA, R.S. LEES, J. BOUYER, J. GILLES et al. (2018). Ecology of reproduction of *Anopheles arabiensis* in an urban area of Bobo-Dioulasso, Burkina Faso (West Africa): Monthly swarming and mating frequency and their relation to environmental factors. PLoS ONE 13(11):e0205966.

CAI Z., Z. YAO, Y. LI, Z. XI, K. BOURTZIS et al. (2018) Intestinal probiotics restore the ecological fitness decline of *Bactrocera dorsalis* by irradiation. Evolutionary Applications 11:1946-1963.

CARTER, N., P. BOULEY, S. MOORE, M. POULOS, J. BOUYER et al. (2018). Climate change, cattle disease, and the future of lions. Conservation Biology 32 (5):1207-1210.

CULBERT, N.J., F. BALESTRINO, H. YAMADA, T. WALLNER, J. BOUYER et al. (2018). A rapid quality control test to foster the development of genetic control in mosquitoes. Scientific Reports 8:16179.

CULBERT, N.J, H. MAIGA, N.S. BIMBILE SOMDA, J.R.L. GILLES, J. BOUYER, W. MAMAI et al. (2018). Longevity of mass-reared, irradiated and packed male *Anopheles arabiensis* and *Aedes aegypti* under mimicked environmental field conditions. Parasites & Vectors 11:603.

DE DEKEN, R. and J. BOUYER (2018). Can sequential aerosol technique be used against riverine tsetse? PLoS Neglected Tropical Diseases 12:e0006768.

DEMIRBAS-UZEL, G., H. KARIITHI, A.G. PARKER, M.J.B. VREYSEN, A.M.M. ABD-ALLA et al. (2018). Susceptibility of tsetse species to Glossina pallidipes salivary gland hypertrophy virus (GpSGHV). Frontiers in Microbiology 9:701.

DEMIRBAS-UZEL, G., A.G. PARKER, M.J.B. VREYSEN, J. BOUYER, A.M.M. ABD-ALLA et al. (2018). Impact of *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV) on a heterologous tsetse fly host, Glossina fuscipes fuscipes. BMC Microbiology. 18(Suppl 1):161.

DEMIRBAS-UZEL, G., A.G. PARKER, M.J.B. VREYSEN, J. VAN DEN ABBEELE, A.M.M. ABD-ALLA et al. (2018). Combining paratransgenesis with SIT: impact of ionizing radiation on the DNA copy number of *Sodalis glossinidius* in tsetse flies. BMC Microbiology 18(Suppl 1):160.

DIARRA, M., M. FALL, A.G. FALL, A. DIOP, J. BOUYER et al. (2018). Spatial distribution modelling of Culicoides (Diptera: Ceratopogonidae) biting midges, potential vectors of African horse sickness and bluetongue viruses in Senegal. Parasites & Vectors 11:341.

DOUDOUMIS, V., A. AUGUSTINOS, A. PARKER, A.M.M. ABD-ALLA, K. BOURTZIS et al. (2018). Different laboratory populations similar bacterial profile? The case of *Glossina palpalis gambiensis*. BMC Microbiology. 18 (Suppl 1):148.

ENGL, T., V. MICHALKOVA, B.L. WEISS, G.D. UZEL, A.M.M. ABD-ALLA et al. (2018). Effect of antibiotic treatment and gamma-irradiation on cuticular hydrocarbon profiles and mate choice in tsetse flies (*Glossina m. morsitans*). BMC Microbiology 18(Suppl 1):145.

EPOPA, P. S., H. MAIGA, D.F.S. HIEN, R.K. DABIRE, R.S. LEES et al. (2018). Assessment of the developmental success of *Anopheles coluzzii* larvae under different nutrient regimes: effects of diet quality, food amount and larval density. Malaria Journal 17:377.

GARROS, C., J. BOUYER, W. TAKKEN and R.C. SMALLEGANGE (2018). Control of vector-borne diseases in the livestock industry: new opportunities and challenges. In: C. Garros, J. Bouyer, W. Takken and R. Smallegange (Eds.). Pests and vector-borne diseases in the livestock industry: Ecology and control of vector-borne diseases. Wageningen Academic Publishers, The Netherlands. p. 575-580.

GEIGER, A., I.I. MALELE, A.M.M. ABD-ALLA and F. NJIOKOU (2018). Trypanosoma-tsetse fly interactions: current and potential future research directions. BMC Microbiology 18 (Suppl 1):162.

GIMONNEAU, G., J.B. RAYAISSE and J. BOUYER (2018). Integrated control of trypanosomosis. In: C. Garros, J. Bouyer, W. Takken and R. Smallegange (Eds.). Pests and vector-borne diseases in the livestock industry: Ecology and control of vector-borne diseases. Wageningen Academic Publishers, The Netherlands. p. 147-174.

HALLMAN, G.J, L. WANG, F. MAXWELL, C.E. CÁCERES BARRIOS, M.J.B. VREYSEN et al. (2018). Comparison of Three Populations of *Bactrocera dorsalis* for Efficacy of Vapor Heat Treatment in Mangoes. Florida Entomologist 101(2):219-222.

HASHEM, A.M., S.S. SOHRAB, S.A. EL-KAFRAWY, A.M.M. ABD-ALLA, S.A. EL-ELA et al. (2018). Diversity of dengue virus-3 genotype III in Jeddah, Saudi Arabia. Acta Tropica 183:114-118.

HASHEM, A. M., S.S. SOHRAB, S.A. EL-KAFRAWY, S.A. EL-ELA, A.M.M. ABD-ALLA et al. (2018). First complete genome sequence of circulating dengue virus serotype 3 in Jeddah, Saudi Arabia. New Microbes and New Infections. 21, 9-11.

HAQ I.U, C. CÁCERES, J.S. MEZA, J. HENDRICHS and M.J.B. VREYSEN (2018). Different methods of methyl eugenol application enhance the mating success of male Oriental fruit fly (Dipera: Tephritidae). Scientific Reports 8:6033.

KARIITHI, H.M., D.G. BOUCIAS, M.J.B. VREYSEN, A.M.M. ABD-ALLA, J.M. VLAK et al. (2018). Coevolution of Hytrosaviruses and Host Immune Responses. BMC Microbiology. 18(Suppl 1):183.

KARIITHI, H.M., I.K MEKI, D.I. SCHNEIDER, V. DEMIRBAS-UZEL, A.M.M ABD-ALLA et al. (2018). Enhancing vector refractoriness to trypanosome infection: achievements, challenges and perspectives. BMC Microbiology 18(Suppl 1):179.

MAMAI, W., L.N. LOBB, N.S. BIMBILE SOMDA, H. MAIGA, H. YAMADA et al. (2018). Optimization of mass-rearing methods for *Anopheles arabiensis* larval stages: Effects of rearing water temperature and larval density on mosquito life-history traits. Journal of Economic Entomology 111:2383-2390.

MARIE, J., D. PERERA, H. GARSTANG, H.C. BOSSIN and K. BOURTZIS (2018). Exploring mosquito fauna of Majuro Atoll (Republic of Marshall Islands) in the context of Zika outbreak. Journal of Medical Entomology, 55:1299-1306.

MASHATOLA, T., C. NDO, L.L KOEKEMOER, L.C DANDALO, K. BOURTZIS et al. (2018). A review on the progress of sex-separation techniques for sterile insect technique applications against *Anopheles arabiensis*. Parasites & Vectors 11 (Suppl 2):646.

MEKI, I.K., İ.A. İNCE, H.M. KARIITHI, A.G. PARKER, A.M.M. ABD-ALLA et al. (2018). Expression Profile of *Glossina pallidipes* microRNAs during symptomatic and asymptomatic infection with Glossina pallidipes salivary gland hypertrophy virus (Hytrosavirus). Frontiers in Microbiology 9:2037.

MEKI, I.K., H.M. KARIITHI, A.G. PARKER, M.J.B. VREYSEN, A.M.M. ABD-ALLA et al. (2018). Hytrosavirus genetic diversity and eco-regional spread in *Glossina* species. BMC Microbiology. 18(Suppl 1):143.

MEKI, I., H.M. KARIITHI, A.G. PARKER, M.J.B. VREYSEN, A.M.M. ABDALLAH et al. (2018). RNA interference-based antiviral immune response against the salivary gland hypertrophy virus in *Glossina pallidipes*. BMC Microbiology 18(Suppl 1):170.

MEZA, J.S., I. Ul HAQ, M.J.B. VREYSEN, K. BOURTZIS, C. CÁCERES et al. (2018). Comparison of classical and transgenic genetic sexing strains of Mediterranean fruit fly (Diptera: Tephritidae) for application of the sterile insect technique. PLoS ONE 13(12):e0208880.

NDO, C., Y. POUMACHU, D. METITSI, J.L.R. GILLES, K. BOURTZIS et al. (2018). Isolation and characterization of a temperature-sensitive lethal strain of *Anopheles arabiensis*. Parasites & Vectors 11 (Suppl 2):659.

NIKOLOULI, K., F. SASSU, C. CÁCERES, R. PEREIRA, K. BOURTZIS et al. (2018). Sterile insect technique and Wolbachia symbiosis as potential tools for the control of the invasive species *Drosophila suzukii*. Journal of Pest Science 91(2):489-503.

ORLOV, I., R. DRILLIEN, D. SPEHNER, M. BERGOIN, A.M.M. ABD-ALLA et al. (2018). Structural features of the salivary gland hypertrophy virus of the tsetse fly revealed by cryo-electron microscopy and tomography. Virology 514:165-169.

PERCOMA, L., A. SOW, S. PAGABELEGUEM, A.H. DICKO, O. SERDEBEOGO, J. BOUYER et al. (2018). Impact of an integrated control campaign on tsetse populations in Burkina Faso. Parasites & Vectors 11:270.

PODA, S., E. GUISSOU, H. MAIGA, J. GILLES, J.B. RAYAISSE, T. LEFEVRE et al. (2018). Impact of irradiation on reproductive performance of field and laboratory *Anopheles arabiensis* mosquitoes. Parasites and Vectors 11:641.

OUEDRAOGO, G.M.S., A. AVGOUSTINOS, A.G. PARKER, M.J.B. VREYSEN, K. BOURTZIS, A.M.M. ABD-ALLA et al. (2018). Prevalence of trypanosomes, salivary gland hypertrophy virus and *Wolbachia* in wild populations of tsetse flies from West Africa. BMC Microbiology 18(Suppl 1):153.

PAPATHANOS, P.A., K. BOURTZIS, F. TRIPET, H. BOSSIN et al. (2018). A perspective on the need and current status of efficient sex separation methods for mosquito genetic control. Parasites & Vectors 11 (Suppl 2):654.

RAMÍREZ-SANTOS, E.M., P. RENDÓN, K. BOURTZIS, C. CÁCERES, A. TARGOVSKA et al. (2018). Evaluation of horizontal gene transfer risk between the Mediterranean fruit fly *Ceratitis capitata* (Tephritidae) and its parasitoid *Fopius ceratitivorus* (Braconidae). PLoS ONE 13(12):e0207999.

SCOLARI, F., G.M. ATTARDO, E. AKSOY, A.M.M. ABD-ALLA, A.G. PARKER et al. (2018). Symbiotic microbes affect the expression of male reproductive genes in *Glossina m. morsitans*. BMC Microbiology 18(Suppl 1):169.

SCHNEIDER, D.I., A.G. PARKER, A.M.M. ABD-ALLA and W.J. MILLER (2018). High-sensitivity detection of cryptic *Wolbachia* in the African tsetse fly (*Glossina* spp.). BMC Microbiology 18(Suppl 1):140.

TAKKEN, W., J. BOUYER, R.C. SMALLEGANGE and C. GARROS (2018). Livestock pests and vector-borne diseases–a much neglected subject. In: C. Garros, J. Bouyer, W. Takken and R. Smallegange (Eds.). Pests and vector-borne diseases in the livestock industry: Ecology and control of vector-borne diseases. Wageningen Academic Publishers, The Netherlands. p. 11-14.

WAMITI, L.G., F.M. KHAMIS, A.M.M. ABD-ALLA, F.L.O. OMBURA, S. SUBRAMANIAN et al. (2018). *Metarhizium anisopliae* infection reduces *Trypanosoma congolense* multiplication in *Glossina fuscipes fuscipes* and its ability to acquire or transmit the parasite. BMC Microbiology 18(Suppl 1):142.

ZACARÉS, M., G. SALVADOR-HERRANZ, D. ALMENAR, R. ARGILÉS, K. BOURTZIS et al. (2018). Exploring the potential of computer vision analysis of pupae size dimorphism for adaptive sex sorting systems of various vector mosquito species. Parasites & Vectors 11 (Suppl 2):656

ZHANG, D., Y. LI, Q. SUN, X. ZHENG, J.R.L. GILLES, H. YAMADA et al. (2018). Establishment of a medium-scale mosquito facility: tests on mass production cages for *Aedes albopictus* (Diptera: Culicidae). Parasites & Vectors 11:189

THE PLANT BREEDING AND GENETICS LABORATORY

EXECUTIVE SUMMARY

Mutation breeding has enabled the development of superior crops with higher yields, tolerance to plant diseases and abiotic stresses such as drought, salinity and heat with a track record of safety for food, feed and the environment. Crop mutation breeding using nuclear techniques is well positioned to help address current and future crop improvement challenges to enhance food security, increase crop resilience and improve adaptation to climate change.

At the Plant Breeding and Genetics Laboratory (PBGL) an integrated breeding approach is followed that incorporates innovations in science and technology to widen the scope of mutation breeding and to improve the efficiency of mutation induction and selection. An important element of PBGL's R&D is to develop protocols and guidelines that can be transferred to the FAO and IAEA Member States for use in field situations, with a socio-economic impact.

PBGL's major achievements in 2018 include: (i) a validated screening protocol for resistance to the parasitic weed *Striga* (*Striga* spp) in sorghum, upland rice and maize, in a controlled greenhouse environment. The protocol provides a practical alternative for field-based screening which is subject to variable environmental conditions and *Striga* infestation levels; (ii) a first marker kit for a feed quality trait in barley - reduced lignin content - validated and successfully applied in the forage barley breeding program of the University of Natural and Life Sciences, Tulln, Austria. Marker-assisted selection for reduced lignin content showed clear genetic gains and increased efficiency compared to phenotypic screening; (iii) using whole genome sequencing and supporting data analysis the chromosomal locus for an early-maturing/semi-dwarf trait was mapped in a mutant farmer-preferred sorghum variety from Sudan. Two F2 populations derived from a cross between two mutant siblings and their parental line, each comprising approximately 250 individuals, and 12,000 F3 progeny plants were planted in the field and phenotyped for height. These experiments showed that the early maturing/semi-dwarf trait is a simple recessive Mendelian trait. A cost-effective protocol for the preparation of whole genome sequencing libraries was developed, along with a cloud-based compute resource for data storage and analysis. The latter effort was carried out jointly with IAEA's MTIT Dept. Building on the successes in barley and sorghum, PBGL's molecular breeding efforts will expand to other priority crops and traits in Member States, such as *Striga* resistance/tolerance, amongst others; (iv) conducting chemical (Ethyl methanesulfonate) mutagenesis of *Coffea arabica* seed and determining LD_{50} values. Previous results from dose-response experiments using gamma-ray irradiation of coffee seed were expanded to include farmer-preferred varieties from Costa Rica; (v) initiating experiments for screening of Cavendish bananas with putative resistance to Fusarium wilt TR4 provided by Dole (Philippines); (vi) contribution to human capacity building of Member States in *in vitro* plant cell and tissue culture and molecular breeding through regional training courses; and (vi) provision of gamma and X-ray crop irradiation services to FAO and IAEA Member States where appropriate facilities are not available.

PBGL's R&D activities are aligned with the following Coordinated Research Projects: CRP D23030 (barley feed applications); CRP D25005 (coffee and banana disease); CRP D25005 (Striga); and CRP D23031 (Improving Resilience to Drought in Rice and Sorghum).

In 2018, the PBGL team published four chapters in the Manual on Mutation Breeding and Biotechnology, 3rd Ed.; a review on Targeting Induced Local Lesions in Genomes as a tool for reverse genetics and mutation discovery; and, contributed to protocols on screening for heat stress tolerance in rice. Further, protocols for mutation induction using X-ray machine sources; coffee mutation induction protocols using physical and chemical mutagenesis; and a genotyping protocol for low-cost, user-friendly detection of Single Nucleotide Polymorphisms (SNP) through allele-specific amplification have been drafted.

In 2018, the PBGL hosted three interns, 14 fellows and seven scientific visitors covering in total 33 man-months training on nuclear techniques for mutation induction, mutant population development and efficiency enhancing biotechnologies. The PBGL organized and implemented one regional training course and one workshop on molecular markers and Next Generation Sequencing and on in vitro plant cell culture as efficiency enhancing techniques for mutation breeding, respectively. The training course and workshop was attended by 30 participants from Asia and the Pacific Islands.

A total of 42 requests for gamma-ray or X-ray irradiation from 30 Member States were received in 2018. These included seven new plant species, confirming the trend of increased interest by Member States for genetic improvement of non-staple crops.

PBGL also welcomed over 700 visitors to the laboratory, showing continued high interest of Member States in crop mutation breeding.

STAFF

Name	Title
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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

A validated protocol for resistance screening to *Striga* parasitic weeds in sorghum, upland rice and maize

The parasitic weeds *Striga asiatica* and *Striga hermonthica* are major biological constraints to cereal production in most of Sub-Saharan Africa and semi-arid tropical regions of Asia. In sub-Saharan Africa, annual losses in cereals due to *Striga* infestation amount to 12 billion US $. *Striga* is particularly challenging because it infects the roots of its host plant and so remains invisible until the time when it emerges from the soil. By that time, the damage to the host plant is already inflicted. Because the damage occurs underground, conventional weed control measures cannot be applied.

The main objective of the CRP D25005 on 'Mutation Breeding for Resistance to *Striga* Parasitic Weeds in Cereals for Food Security' is to develop laboratory, screenhouse and field screening protocols of mutant populations of sorghum and upland rice for resistance to *Striga asiatica* and *Striga hermonthica*. In addition, the CRP focuses on technologies such as rapid cycling of generation of crop plants, doubled haploid techniques and molecular markers to enhance the efficiency of mutant identification and accelerate delivery of resistant varieties.

In continuation of the ongoing R&D activities related to this CRP, the PBGL has conducted verification trials using the established protocol for screening of mutant populations for resistance to *Striga spp.* A protocol for pot-screening was optimized using varieties of known reaction to *Striga* (resistant vs susceptible) both in sorghum and upland rice and validated on an M_2 population of sorghum. The protocol was used to verify 19 putative mutants of maize (M_4–M_5) and 12 putative mutants of upland rice identified by the CRP contract holder in Madagascar. The mutants were tested against both *Striga asiatica* from Madagascar and *Striga hermonthica* from Sudan. *Striga* seeds (0.05g) were placed in medium-size pots filled with soil mixture. Four pots were assigned for each mutant per each of the two *Striga* treatments. Wildtype parents were included for each crop in four pots with and without *Striga* seeds. Plants were maintained in a glass-house under 25° Celsius and natural light at the PBGL facility in Seibersdorf, Austria. Seedlings were irrigated two times a week until establishment of 4–6 leaf stage and then continued with one watering per week. No fertilizer was applied. *Striga* plants started to emerge above the soil in about 40 days in *S. hermonthica* and 60 days in *S. asiatica* treatments. After three months, the experiment was uniformly covered with *Striga* plants at different stages including many flowering plants (Figure 1). The damage became clear on the host plants with many completely dead in the *S. hermonthica* treatments. Overall, all the evaluated mutants in maize and rice were infected by *S. hermonthica* while there was variation among mutants in their reactions (resistance/tolerance) to *S. asiatica*. Among the tested maize mutants, 9, 8 and 2 were resistant, tolerant and susceptible to *S. asiatica* respectively, while 6, 4 and 2 of the tested rice mutants were resistant, tolerant and susceptible. Seeds were collected for further advanced analyses to identify their allelism and the underlying mechanism of resistance.

These results clearly prove the efficiency and accuracy of the established protocol in verifying putative mutants. Our work will continue to verify other putative mutants identified by the CRP contract holders from Burkina Faso and Sudan. Few (three from each crop/participating country) of the most consistent mutants will be chosen for development of molecular markers to facilitate introgression and pyramiding to produce superior resistant varieties in the respective crops by Member States.

Fig. 1. Screening experiment showing variation in the number of Striga plants per pot while in one pot no Striga plants have emerged suggesting that this M₂ plant is a putative resistant mutant

Mutant trait discovery and marker development for dissemination of priority mutant traits to Member States

At PBGL, an integrated breeding approach is used for mutant trait discovery and to associate priority mutant traits with the underlying causative induced mutations. Both a candidate gene approach and Next Generation Sequencing techniques (NGS) for whole genome sequencing are followed. Using NGS and accurate phenotyping, genetic mapping and marker-trait connections can be made in unprecedented short amount of time. However, to reap the benefits of the low-cost sequencing, clever genetics, cost-effective molecular biology protocols, and data analysis capacity are required.

In 2018, a first marker kit for a feed quality trait in barley has been validated and successfully applied in a forage barley breeding program of a Member State, Austria. The causative mutations for reduced lignin content in barley were identified using a candidate gene approach. Further, the team at PBGL made significant progress by developing high-throughput DNA sequencing library production protocols and establishing compute resources for whole genome sequencing data analysis. It applied new protocols on segregating populations on farmer-preferred sorghum from Sudan, grown in the Seibersdorf greenhouse and field, and determined a locus on Chromosome 4 to be responsible for an early maturing/semi-dwarf trait. The next steps will be to convert the closest linked DNA sequence variant into a marker assay and to validate the marker in different sorghum genetic backgrounds to verify its wider use for marker-assisted breeding of early-maturing/semi-dwarfism in sorghum.

Why are marker assays useful?

Genotyping an individual for a molecular marker linked to a trait allows for predicting whether this individual will exhibit the trait or not. This is very powerful, especially when the trait itself can only be observed in late stages of development and/or when the phenotyping method is expensive or complicated (Ingelbrecht et al., 2018). Imagine a fruit trait in a coffee plant. Coffee plants can take up to three years to bear fruits. Being able to select them at the seedling stage saves a lot of space and work. In addition, modern marker assays can be designed to be *co-dominant,* which means that they also classify the *heterozygous* individuals. This is important because most traits induced using nuclear techniques are *recessive*, which means that the trait is not visible in *heterozygous* individuals. With the help of a molecular marker these traits can be exposed, and this will allow the breeder to skip entire generations thus saving precious time in breeding programs and shortening the breeding cycle.

A first marker kit for a feed quality trait in barley – reduced lignin - validated and successfully applied in an elite forage barley breeding program

Improving feed quality is an important breeding goal for barley which is widely used as feed for animals. We previously reported the development of a molecular marker assay for the Orange Lemma (OL, *rob1*) trait in the barley Bowman genetic background. OL results in reduced lignin content which is beneficial for feed applications.

The assay was adapted for low-cost, user-friendly detection on standard agarose gels to facilitate technology transfer to Member States with basic molecular biology infrastructure. The assay enables detection of the wild type and Y28 mutant allele in the *cad2* gene and thus can identify heterozygous individuals that do not express the trait. Genetic analysis showed that the OL trait is a simple, recessive trait.

In 2018, the marker assay was validated through genotyping and phenotyping an F2 segregating population, showing perfect association between the OL phenotype and the marker (Figure 2).
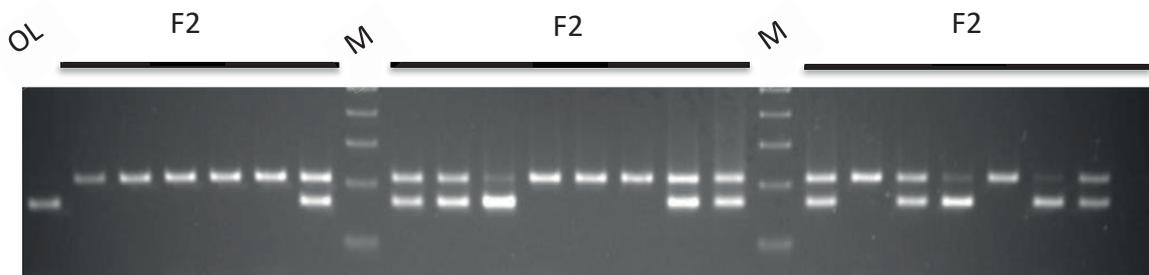

Fig. 2. Marker assay on F2 progeny showing segregation of wild type and mutant alleles. OL is Orange Lemma; M is the size marker. Upper band is wild type allele; lower band is mutant (Y28) allele.

Subsequently, the marker assay was tested in a forage barley program of Prof. Grausgruber, BOKU, Austria, for its potential to predict the reduced lignin phenotype when introduced into Verdant, an elite winter barley variety. Verdant is a 'hooded' mutant where the awn is replaced by an inverted floret and is useful for animal feed applications (Figure 3). Prof. Grausgruber's barley breeding program is aimed at further improving the feed quality of Verdant by stacking 'hooded' with the *rob1* reduced lignin trait. The marker assay correctly predicted the *rob1* phenotype in a segregating F2 population derived from a cross between Verdant and Bowman *rob1*. This result extends the use of the marker kit to unrelated genetic background such as Verdant, thus opening perspectives for wider applications in Member States with barley fodder breeding programs.

Fig. 3. Barley field trials. Inset is 'hooded' mutant barley which is being combined with Orange Lemma using PBGL's kit for marker-assisted selection for feed quality improvement.

Chromosomal locus harbouring the causative mutation for early-maturing/semi-dwarf trait in sorghum identified using whole genome sequencing

As explained above, molecular markers linked to traits will enable faster introgression and wider utilization of the trait by Member States. PBGL is developing protocols to enable Member States to efficiently use Next Generation Sequencing techniques (NGS) to establish phenotype to genotype associations. We are developing those genomic tools along a pilot experiment on Sorghum to establish a comprehensive fast forward genetics approach to genetically map causal loci in a cost-effective manner. In case of our pilot study on Sorghum, the segregation pattern of the trait revealed that there very likely is one causal locus. The semi-dwarf/early-maturing trait segregates like a classical mendelian, recessive locus with a ratio of 1 in 4.

Wad Ahmed is a popular sorghum variety among farmers in Sudan, but it matures slightly late and is tall, which makes it prone to yield losses by terminal drought and lodging. We previously identified six promising mutants that exhibited a smaller stature and matured earlier than wild-type. These mutants, numbered D1 through D6, were progressed to the M_6-stage by single seed descent.

For genome-wide identification of candidate mutations, we produced 2nd-generation DNA sequencing data by Illumina DNA sequencing for all six mutagenized lines (D1-D6, M_6-generation) and for wild-type *Wad Ahmed* plants. To compare mutants with wild-type, we aligned the sequencing reads to the publicly available Sorghum reference genome (v3.10). We then searched for differences between our mutants and wild-type *Wad Ahmed* and detected hundreds of small variants such as SNPs and InDels (Figure 4) plus some large structural variants; most pronounced is a large, six Megabase-long deletion close to the Centromere on Chromosome 9 (Figure 5).
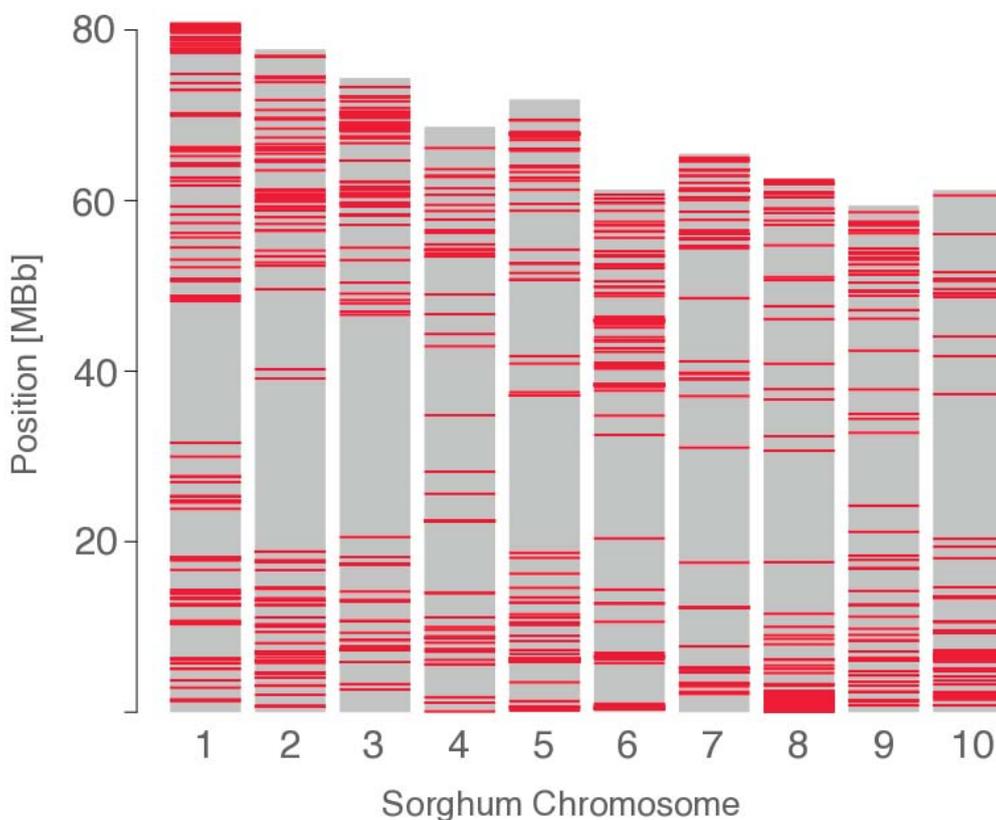

Fig. 4. *Distribution of small DNA variants: SNPs and small Insertions and Deletions (InDels) across the ten sorghum chromosomes as identified by whole-genome sequencing of six mutant lines and compared to the parental variety.*

Sorghum Chromosomes
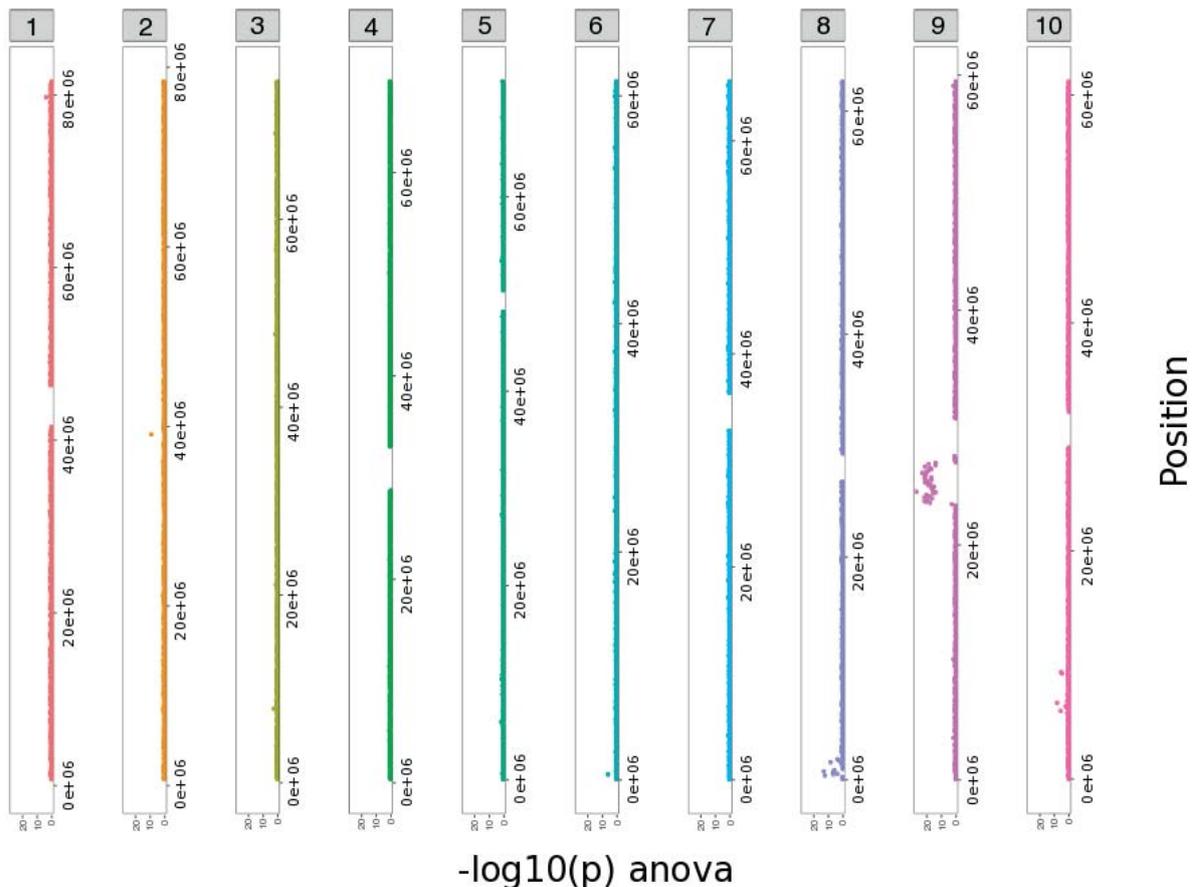
Fig. 5. Read coverage analysis along the 10 chromosomes against the Sorghum reference genome identifies large structural variants shared by the six mutants. Most pronounced is a six MegaBase-long deletion close to the centromere on Chromosome 9 (2nd from the right). Further analysis revealed that it is not involved in the mutant phenotype.

For marker development, we will need to pin-point those variants that are closely linked to the trait; ideally determine the causal mutation. The variants that we have identified so far are illustrated in Figures 4 and 5. There are way too many to take a guess as to which of them is the causal one!

We hence performed a Bulk Segregant Analysis to genetically map the locus. *Genetic mapping* describes a set of techniques that can identify links between variants (the genotype) and the phenotype by exploring co-segregation of the trait with the variant in segregating populations. The underlying principle why this works is *genetic linkage*. Linkage decays from one generation to the next through recombination, where closely linked pieces of DNA have a higher probability of being inherited together, i.e., to co-segregate.

For Bulk Segregant Analysis, one simply bulks the individuals of contrasting phenotypes into separate pools, and then looks which mutation also gets separated into the different pools along with the trait. In our case we pooled small F2 plants into the "dwarf" pool and tall, normal sized ones into the "tall" pool.

These pools were then sequenced by Next Generation Sequencing. Due to genetic linkage and given enough individuals per pool, the expectation in such Bulk Segregant Analysis is that mutations that are not associated with the trait are randomly assorted to each of the two pools, while the mutation that is causing the phenotype is enriched in one, but absent in the other. In our case the mutation causing the "dwarfism" will be enriched in the pool with the "dwarf" plants but should be absent in

the pool with the "tall" plants. We found this to indeed be the case and currently have a handful of very strong candidates in a small interval in the middle of chromosome 4.

We are currently preparing a publication which will provide the details on the procedure and the results. We can report at this stage that PBGL has established the molecular biology protocols and data analysis capacities that are needed to cater for cost-effective fast forward genetic studies using Next Generation Sequencing. The techniques and protocols are applicable to a large variety of experimental settings and we can offer this capacity to joint projects with Member States.

Early maturity is an important secondary trait for avoiding drought conditions, for example in case of early onset of the dry season in tropical regions. Hence, this project links with CRP D23031 on 'Improving Resilience to Drought in Rice and Sorghum through Mutation Breeding'. The protocols are also useful in the context of CRP D25005 on 'Mutation Breeding for Resistance to *Striga* Parasitic Weeds in Cereals for Food Security'.

Protocol development for EMS mutagenesis of *Coffea arabica* mature seed

Dose optimization experiments were conducted for EMS mutagenesis of a commercial variety of *Coffea arabica* from Costa Rica 'Venecia'. A total of 50 mature seed were treated with the following EMS doses: control (0%); 0.2%; 0.5%; 1%; 2%; 4% and 6%. Treated seed was sown in trays and grown under natural daylight conditions in the greenhouse. The parameters used for determining LD_{50} values of the treated seed were % germination and % survival relative to the values obtained for the control, untreated seed. The germination frequency of the control was 56%. Final measurements were taken four months after sowing. The LD_{50} for mature seed was determined at 4% EMS (see Figure 6).
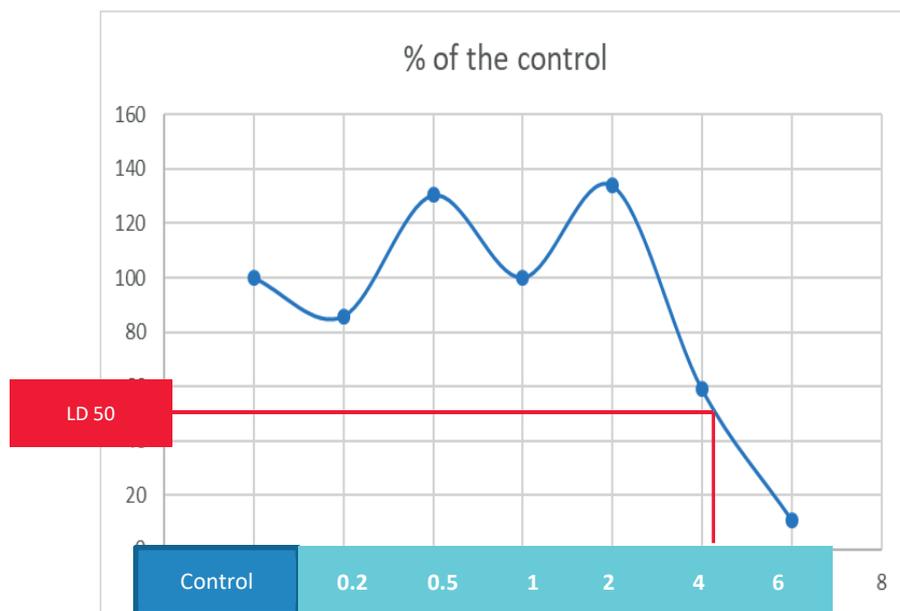
Fig. 6. *EMS dose response experiments of mature seed of Coffea arabica, cv Venecia*

Similar experiments were conducted for gamma-ray irradiation of mature seed of three *Coffea arabica* varieties: Venecia, Catuai and Caturra. Following doses were tested: 0; 20; 50; 100; 150; 200; 250; 300; and 350 Gy. The LD_{50} value for Venecia, Catuai and Caturra was 50, 80 and 90 Gy, respectively (see Figure 7). The in-house gamma cell irradiator (with ^{60}Co source) was used in these experiments with a dose rate of ca 140 Gy/min.
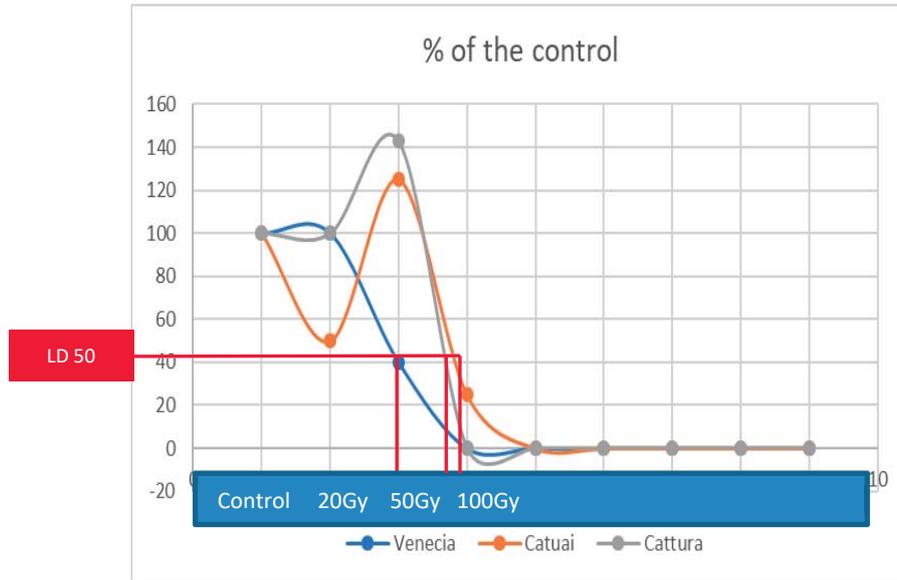
Fig. 7. *Gamma-ray radiosensitivity testing of Coffea arabica mature seed, cv Venecia, Catuai and Cattura*

Progress towards a screening protocol for resistance to Fusarium Wilt TR4 in Cavendish banana

Four putative Foc TR4 resistant banana clones obtained from the CRP counterpart in the Philippines are being mass propagated *in vitro* at the PBGL. Inoculation experiments have been initiated to verify their resistance phenotype as compared to susceptible controls in pot experiments (Figure 8) and under in vitro conditions. The following parameters are being tested: age of the plant, soil composition, and inoculum concentration of the pathogen. Preliminary results show that the pot experiments provide more reliable results compared to the in vitro experiments due to excessive growth of the pathogen under the in vitro conditions causing necrosis and cell death of both the susceptible and resistant control plants.
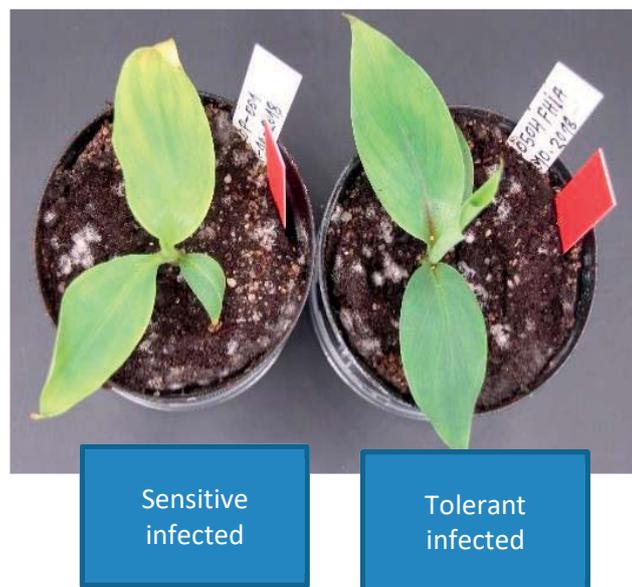
Fig. 8. *Inoculation experiments to screen putative TR4 resistant mutant Cavendish banana (right) as compared to susceptible control (left)*

CAPACITY BUILDING

Regional Training Workshop on Mutation Breeding and supportive biotechnologies for Crop improvement in the Pacific Islands, RAS5079, Seibersdorf, Austria, 16-25 May 2018

A nine-day training workshop was organized on mutation breeding and supportive biotechnologies for crop improvement in the Pacific Islands under the Regional TC Project RAS5079 "Improving crops resilience to climate change through mutation breeding in Pacific Islands". It was delivered to eight participants from the Pacific Islands (Fiji, Marshall Islands, Papua New Guinea), one MSc student from Indonesia and a Scientific Visitor from Nicaragua. In addition to the staff of the PBGL, two external experts provided lectures and practical sessions during the training: Em. Prof. Rajbir Singh Sangwan (Université de Picardie Jules Verne, UFR Sciences, France) and Prof. Stefaan Werbrouck (University Ghent, 9000 Ghent, Belgium). The course covered principles of mutation Induction, the development of mutant populations and the use of efficiency enhancing technologies with a focus on the use of in vitro plant cell and tissue culture techniques for mutation breeding of vegetatively propagated plants relevant to the Pacific Islands such as taro, sweet potato, yam, cassava and trees such as breadfruit. The participants presented their workplan and had a very useful discussion on the most relevant *in vitro* techniques for the targeted plant species in their respective project.

Regional Training Course on Molecular Techniques Applied in Mutation Breeding, RAS5073, Seibersdorf, Austria, 19–29 June 2018

New DNA sequencing and marker genotyping technologies provide excellent opportunities to accelerate mutation breeding programs and to widely disseminate priority traits for Member States. Next Generation Sequencing (NGS) technologies are allowing massively parallel sequencing of genomes which allows to discover new genes and their positions, makes available large collections of molecular markers and permits the identification of markers linked to the genes and QTLs. These markers can be used for marker assisted selection, including marker assisted backcross selection or genomic selection.

The regional training course was organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture under the TC RAS5073 on Supporting Climate-Proofing Rice Production Systems (CRiPS) Based on Nuclear Applications. Fifteen RAS5073 participants from Bangladesh, Cambodia, China, Indonesia, Lao PDR, Malaysia, Mongolia, Myanmar, Nepal, Pakistan, Philippines, Thailand and Viet Nam attended the training course. In addition, the training course was attended by six Scientific Visitors and fellows from three different countries.

The training course covered molecular breeding techniques with specific focus on applications of molecular markers and Next Generation Sequencing (NGS) for mutation breeding of self-pollinated crops. The course consisted of practical experiments and lectures in genetics and molecular techniques, including latest advances in DNA sequencing and bioinformatics tools for the characterization of mutant traits. Approaches for the development and use of molecular markers for plant mutation breeding were also covered.

In addition to the PBGL staff, Dr. Tilak Raj Sharma, National Agri-Food Biotechnology Institute, India, provided lectures illustrating the development and application of molecular markers for blast resistance breeding in rice.

Fellowships, Scientific Visitors and Interns

The PBGL hosted 14 fellows (F), eight scientific visitors (SV), two interns and one MSc-consultant who were trained in the following topics:

Name	Country	Status	Topic	Period
Mr Guillermo REYES CASTRO	Nicaragua	SV	*In vitro* tissue culture	10 days
Mr Andrew PEARSON	Jamaica	SV		1 day
Ms Huijun GUO	China	SV	FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology	5 days
Mr Luxiang LIU	China	SV	FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology	5 days
Mr Ham Huy LE	Viet Nam	SV	FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology	5 days
Mr Thao Duc LE	Viet Nam	SV	FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology	5 days
Mr Hoi Xuan PHAM	Viet Nam	SV	FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology	5 days
Ms Aisha AL-KUWARI	Viet Nam	SV	FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology	5 days
Mr Seyni BOUREIMA	Niger	F	*Striga* screening protocol, marker development; EMS mutagenesis pearl millet	2 months
Mr Hamissou ZANGUI	Niger	F	Allelic diversity of key traits for sesame breeding, marker development	2 months
Mr. H. Jhonny RABEFIRAISANA	Madagascar	F	*Striga* screening protocol, marker development	7 months

Name	Country	Status	Topic	Period
Mr. Ryan FRANCIS	Jamaica	F	Mutation induction in vegetatively propagated plants	3 months
Ms. Heeidy COREA NARVAEZ	Nicaragua	F	Mutation induction in vegetatively propagated plants	3 months
Mr. Lameck NYALIGWA	Tanzania, United Republic of	F	Mutation induction and detection	2 weeks
Mr. Francis Nabieu LASSAYO	Sierra Leone	F	Mutation induction and detection	1 month
Ms. Habibah AL-MENAI	Kuwait	F	Mutation induction and detection	2.5 months
Ms. Ngoyi CHIBALANGE	Zambia	F	Molecular marker development in barley	3 months
Mr. Faiz AHMAD	Malaysia	F	Mutation induction and detection using NGS techniques	3 months
Mr. Mustapha AKIL	Malaysia	F	Mutation induction and detection using NGS techniques	3 months
Mr. Saleh Ali Said AL HINAI	Oman	F	Mutation induction and detection	3 months
Mr. Ahmed Said Ali AL-MAWALI	Oman	F	Mutation induction in vegetatively propagated plants	3 months
Mrs. Donnette JACKSON-HOWELL	Jamaica	F	Mutation induction in vegetatively propagated plants	3 months
Ms Samira TAJEDINI	Iran	I	Haploid in rice and sorghum; mutant population development *Striga*	7 months
Mr Edwin THEKKINEN	Austria	I	Molecular markers for trait discovery in barley	6 months

PLANT IRRADIATION SERVICES

In 2018, the PBGL received a total of 42 requests for plant irradiation from 30 Member States, covering 28 different plant species. These included requests for seven new plant species. Twenty-seven requests were received in the context of CRPs, TCPs or fellowships (F) while the remaining requests were from stakeholder institutions from Member States, as summarized in the table below. Under the new 2018–2019 TC project cycle, PBGL received several requests for vegetatively propagated crops, including taro, cocoyam and sweet potato as well as trees/perennial crops such as eucalyptus, coffee, and breadfruit. In 2018, the PBGL started to jointly carry out the radio-sensitivity testing with the requesting institutions in the country of origin, with guidance and technical backstopping provided by the PBGL. The total number of irradiation requests now stands at 1582.

Request Number	Country	Request Type	Plant
1541	United Kingdom		Wheat
1542	Hungary		Ornamental
1543	Austria	CRP	Coffee
1544	France/ Vanuatu	TC	Sweet Potato
1545	Nepal	TC	Sweet Potato
1546	Ireland		Eucalyptus
1547	Mongolia	TC	Wheat, Barley, Rape
1548	Germany		Ornamental
1549	Kazakhstan	TC	Wheat
1550	Togo	TC	Rice
1551	Niger	TC	*Neocarya macrophylla*
1552	The Netherlands		Ornamental
1553	Austria, PBGL	CRP	Coffee, Petunia
1554	Congo, Democratic Republic of	TC	Soybean
1555	The Netherlands		Ornamental
1556	Hungary		Ornamental
1557	Czech Republic		*Papaver somniferum*
1558	Mongolia	TC	Rape

Request Number	Country	Request Type	Plant
1559	Fiji	TC	Sweet Potato, Yam, Breadfruit
1560	Germany		Ornamental
1561	Vanuatu	TC	Sweet Potato
1562	Cambodia	TC	Rice
1563	PBGL	F	Rice
1564	PBGL		Sorghum
1565	Jamaica	TC	Ginger
1566	Nicaragua	F	*Xsanthosoma violaceum, Xsanthosoma sagittifolium*
1567	United Kingdom		*Alopecurus myosuroides*
1568	Senegal	TC	Cowpea
1569	Kenya	TC	Dolichos, Wheat, Rice, Maize
1570	Namibia	TC	Sorghum
1571	Palestine	TC	Barley
1572	Zimbabwe	TC	Common Bean
1573	Namibia	TC	*Vigna subterranea, Cleome gynandra*
1574	Kuwait	TC	Barley
1575	Ireland		*Eucalyptus sp.*
1576	Malawi	TC	Maize, Soya, Groundnut
1577	Zimbabwe	TC	*Vigna subterranea, Cicer arietinum*
1578	Chile	TC	*Camelina sativa, Linum usitatissimum*
1579	Namibia	TC	Cowpea, Bambara groundnut
1580	Germany		ornamental
1581	Austria		maize
1582	Bangladesh		wheat

INFORMATION DISSEMINATION

Infographic on Marker-Assisted Plant Breeding

Markers and Marker-Assisted-Selection (MAS) are important for efficient plant breeding. To support a growing understanding of these techniques and how they can be used in mutation breeding, PBG together with FAO developed an Infographic. It is available online on the Joint FAO/IAEA Programme's Multimedia page:

http://www-naweb.iaea.org/nafa/resources-nafa/multimedia.html#

Direct link to the infographic:

http://www-naweb.iaea.org/nafa/resources-nafa/Marker-Assisted-Plant-Breeding-ST-English-web.mp4

Video featuring Marker-Assisted Selection for forage barley breeding

At the IAEA Ministerial Conference on Nuclear Science and Technology (27–30 November 2018, Vienna, Austria), a film showing five innovative techniques related to nuclear applications, including the use of molecular markers for forage barley breeding, was shown. The film is available online on the IAEA website https://www.iaea.org/events/ministerial-conference-on-nuclear-science-and-technology-2018.

PBGL outreach materials

In 2018, the PBGL team produced a new folder with flyers summarizing PBGL's R&D activities (see Figure 9) which were widely disseminated at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology (27–31 August 2018), the Ministerial Conference (28–30 November 2018) and to many visitors and meeting participants.

Fig. 9. *PBGL outreach materials*

PUBLICATIONS

Protocols and Guidelines

Physical mutagenesis. Spencer-Lopes, M.M., Jankuloski L., Mukhtar Ali Ghanim, A., Matijevic, M. and Kodym, A. Chapter 1 in Manual on Mutation Breeding and Biotechnology, 3rd Ed. (2018). M Spencer-Lopes et al. (eds.). Co-published by the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization (FAO) of the United Nations. http://www.fao.org/3/I9285EN/i9285en.pdf; ISBN 978-92-5-130526-3. © FAO, 2018

Chemical mutagenesis. Ingelbrecht I., Jankowicz-Cieslak J., Till B.J., and Szarejko, I. Chapter 2 in Manual on Mutation Breeding and Biotechnology, 3rd Ed. (2018). M Spencer-Lopes et al. (eds.). Co-published

by the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization (FAO) of the United Nations. http://www.fao.org/3/I9285EN/i9285en.pdf; ISBN 978-92-5-130526-3. © FAO, 2018

Mutation Breeding in seed propagated crops: parental selection, mutant generation development, mutation detection, mutant evaluation and factors influending success. Mukhtar Ali Ghanim, A., Spencer-Lopes, M.M., and Thomas, W. Chapter 5 in Manual on Mutation Breeding and Biotechnology, 3rd Ed. (2018). Eds M Spencer-Lopes, B.P. Forster and L. Jankuloski. Co-published by the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization (FAO) of the United Nations. http://www.fao.org/3/I9285EN/i9285en.pdf ; ISBN 978-92-5-130526-3. © FAO, 2018

DNA marker and genotyping applications for mutation breeding. Ingelbrecht I., Jankowicz-Cieslak J., Mukhtar Ali Ghanim, A., Anwar Y., Forster, B.P. and Till B.J. Chapter 8 C in Manual on Mutation Breeding and Biotechnology, 3rd Ed. 2018. M Spencer-Lopes et al. (eds.). Co-published by the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization (FAO) of the United Nations. http://www.fao.org/3/I9285EN/i9285en.pdf ; ISBN 978-92-5-130526-3. © FAO, 2018

Pre-Field Screening Protocols for Heat-Tolerant Mutants in Rice. Sarsu, F., Ghanim, A.M.A., Das, P., Bahuguna, R.N., Kusolwa, P.M., Ashraf, M., Singla-Pareek, S.L., Pareek, A., Forster, B.P., Ingelbrecht, I. (2018). Springer. ISBN 978-3-319-77338-4. https://www.springer.com/de/book/9783319773377

TILLING: The Next Generation. Till, B.J., Datta, S., and Jankowicz-Cieslak J. (2018) In: Advances in Biochemical Engineering/Biotechnology. Rajeev K. Varshney et al. (eds.), Springer.

DOI: 10.1007/10_2017_54

Peer-reviewed Journal Articles

AMRI, W.T., LAOUAR, M., ABDELGUERFI, A., JANKOWICZ-CIESLAK, J., TILL, B.J. (2018) Genetic Variability Induced by Gamma Rays and Preliminary Results of Low-Cost TILLING on M_2 Generation of Chickpea (*Cicer arietinum* L.). Frontiers in Plant Science-Plant Breeding 9:1568. DOI: 10.3389/fpls.2018.01568

DATTA, S., JANKOWICZ-CIESLAK. J., NIELEN, S., INGELBRECHT, I., TILL, B.J. (2018) Induction and Recovery of Copy Number Variation in Banana through Gamma Irradiation and Low Coverage Whole Genome Sequencing. Plant Biotechnology Journal. DOI: 10.1111/pbi.12901

GRONENBORN, B., RANDLES, J.W., DENNIS KNIERIM, BARRIÈRE, Q., VETTEN, H.J., WARTHMANN, N., CORNU, D., SILEYE, T., WINTER, S., TIMCHENKO, T. (2018) Analysis of DNAs Associated with Coconut Foliar Decay Disease Implicates a Unique Single-Stranded DNA Virus Representing a New Taxon. Scientific Reports 8, Article number: 5698. DOI: 10.1038/s41598-018-23739-y

ZHANG, C, SIMPSON, R.J., KIM, C.M., WARTHMANN, N., DELHAIZE, E., DOLAN, L., BYRNE, M.E., WU, Y., RYAN, P.R. (2018) Do Longer Root Hairs Improve Phosphorus Uptake? Testing the Hypothesis with Transgenic *Brachypodium distachyon* Lines Overexpressing Endogenous RSL Genes. New Phytol. 2018 Jan 17; 157:97.

TRAMONTANO, A., JARC, L., JANKOWICZ-CIESLAK, J., HOFINGER, B.J., GAJEK, K., SZURMAN-ZUBRZYCKA, M., SZAREJKO, I., INGLEBRECHT, I., TILL, B.J. (2018) Fragmentation of pooled PCR products for deep amplicon sequencing. bioRxiv preprint first posted online 24 October 2018. http://dx.doi.org/10.1101/451567

BACHIRI, H., DJEBBAR, R., MEKLICHE, A., DJENADI, C., GHANIM, A.M.A. (2018). Carbon Isotope Discrimination as a Physiological Marker to Select Tolerant Wheat Genotypes (*Triticum aestivum* L.) Under Water Limited Conditions. American Journal of Plant Physiology SSN 1557-4539, DOI: 10.3923/ajpp.2018.1-7

KAMAL, N.M., MOHAMMED, Y., GHANIM, A.M.A. (2018) Performance of Sorghum Stay-green Introgression Lines Under Post-Flowering Drought. April 2018. International Journal of Plant Research 7(3). DOI: 10.5923/j.plant 20170703.02

KAMAL, N.M., ALNOR GORAFI, Y.S., TSUJIMOTO, H., GHANIM, A.M.A. (2018) Stay-Green QTLs Response in Adaptation to Post-Flowering Drought Depends on the Drought Severity. BioMed Research International Volume 2018, Article ID 7082095. https://doi.org/10.1155/2018/7082095

Conference Abstracts and Posters

ENAYATI SHARIATPANAHI, M., TAJEDINI, S., GHANIM, A.M.A., FAKHERI, B., OROOJLOO, M., MAHDINEJAD, N. (2018) Haploidy in Rice (*Oryza sativa* L.) Mutation Breeding for *Striga* Resistance. Poster 79 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

ENAYATI SHARIATPANAHI, M., TAJEDINI, S., GHANIM, A.M.A., FAKHERI, B., OROOJLOO, M., MAHDINEJAD, N. (2018) Enhancing Efficiency of Mutation Breeding for *Striga* resistance in Sorghum by Haploid Technology. Poster 82 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

MUNASINGHA JAYASUNDARA MUDIYANSELAGE PRIYANTHI KUMARARATHNA, GHANIM, A.M.A. (2018) Mungbean Radiosensitivity Test to Gamma Irradiation for Mutation Breeding in Mungbean. Poster 100 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

SARAYE, B., JANKOWICZ-CIESLAK, J., PEERBOCCUS, S., TILL, B.J., NOWBUTH, R.D. (2018) Induced Genetic Variability for Yield and Heat Tolerance in Tomato (*Solanum lycopersicum*). Poster 111 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

MAGHULY, F., JANKOWICZ-CIESLAK, J., GUPTA, P., TILL, B.J., LAIMER, M. (2018) Creation and Characterization of an EMS Mutant Population of *Jatropha curcas*. Abstract 184 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

SARAYE, B., JANKOWICZ-CIESLAK, J., TILL, B.J. (2018) A Pre-Screening Methodology for Identification of Heat Tolerant Mutant in Tomato (*Solanum lycopersicum* L.). Poster 254 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

DUSSORUTH, B., JANKOWICZ-CIESLAK, J. (2018) Irradiation as Means for Increasing Genetic Diversity in Banana: The Need to Carry Out Radiosensitivity Tests on *In Vitro* Cultures at Appropriate Stage of Growth. Poster 255 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

HASSAN, O., GHANIM, A.M.A., INGELBRECHT, I. (2018) Mutation Induction in Pearl Millet (*Pennisetum glaucum*) and Fingermillet (*Eleusine coracana*) for Drylands in Sudan. Poster 257 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

LAOUAR, M., TILIOUINE AMRI, W., DAHBIA, T., JANKOWICZ-CIESLAK, J., TILL, B.J. (2018) Early Assessment of Lentil and Chickpea Mutant and Evaluation of Low Cost TILLING on M_2 Chickpea. Abstract 269 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

SARSU, F., GHANIM, A.M.A., PAREEK, A. FORSTER, B.P., INGELBRECHT, I., ASHRAF, M., KUSOLWA, P.M., DAS, P., BAHUGUNA, R., SINGLA-PAREEK, S.L. (2018) Screening Protocols for Heat Tolerant Mutants in

Rice. Poster 273 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

TRAMONTANO, A., JARC, L., JANKOWICZ-CIESLAK, J., HOFINGER, B.J., GAJEK, K., SZURMAN-ZUBRZYCKA, M., SZAREJKO, I., INGLEBRECHT, I., TILL, B.J. (2018) Fragmentation of Pooled PCR Products for Deep Amplicon Sequencing. Poster 286 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

JANKOWICZ-CIESLAK, J., GOESSNITZER, F., CHAO, C.-P., HUANG, S.-H., DATTA, S., INGELBRECHT, I., TILL, B.J. (2018) Induced Mutagenesis for Generating Bananas Resistant to Fusarium Wilt TR4. Poster 287 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology 27–31 August 2018, Vienna, Austria.

JANKOWICZ-CIESLAK, J., HOFINGER, B.J., JARC, L., JUNTTILA, S., GYENESEI, A., INGELBRECHT, I., TILL, B.J. (2018) Mapping the Landscape of Gamma and X-ray-Induced Mutations in Rice. Poster 288 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

JANKOWICZ-CIESLAK, J., SARAYE, B., JUNTTILA, S., GYENESEI, A., INGELBRECHT, I., TILL, B.J. (2018) Whole Genome Sequencing of Advanced Mutant Lines of Heat Tolerant Tomato Induced by Gamma Irradiation. Poster 289 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

TILL, B.J., JANKOWICZ-CIESLAK, J., HUYNH, O., HOFINGER, B., ATTA, S., TRAMONTANO, A., JARC, L., NIELEN, S., FORSTER, BP., MBA, C., INGELBRECHT, I. (2018) Genomics Tools to Facilitate Plant Mutation Breeding. Abstract 290 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

GHANIM, A.M.A., SNIGDHA, R., JANKULOSKI, L., INGELBRECHT, I. (2018) Pre-Field Phenotyping of Lentil Mutants for Drought Tolerance Using Polyethylene Glycol. Poster 291 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

GHANIM, A.M.A., MUSTAFA, N.S., JANKULOSKI, L., INGELBRECHT, I. (2018) Optimization of doubled haploid production for enhancing efficiency of wheat mutation breeding. Poster 292 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

GHANIM, A.M.A., SEN, A., SHIRAZI, F.T., JANKULOSKI, L., INGELBRECHT, I. (2018) Rapid Cycling Techniques to Accelerate Plant Mutation Breeding in Cereals. Poster 293 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

GHANIM, A.M.A., ABDUL MALEK, M., JANKULOSKI, L., INGELBRECHT, I. (2018) Optimization of screening for salt tolerance in soybean for mutation breeding. Poster 294 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

GHANIM, A.M.A., DADA, K., INGELBRECHT, I. (2018) Irradiation-dose optimization for mutation induction in coffee. Poster 295 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

GHANIM, A.M.A., ALI, A., JANKULOSKI, L., INGELBRECHT, I. (2018) Mutation breeding for resistance to the parasitic weed *Striga* in major cereal crops for sustainable food security. Poster 296 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

INGELBRECHT, I., HOFINGER, B., AKGUN, E., MATIJEVIC, M., ALI, A., JANKOWICZ-CIESLAK, J., JARC, L., JANKULOSKI, L., GHANIM, A.M.A., GRAUSGRUBER, H. (2018) Development of a functional marker for marker-assisted selection of 'Orange Lemma' mutants to improve feed quality in barley. Poster 298 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

WARTHMANN, N., GHANIM, A.M.A., ALI, A., MATIJEVIC, M., JANKOWICZ-CIESLAK, J., INGELBRECHT, I. (2018) Creating desired traits for African sorghum, semi-dwarf and early maturing, and molecular characterisation of their genetic architecture. Poster 299 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

RAKOTOARISOA, N.V., GHANIM, A.M.A., ANDRIANJAKA, A., RASOAMAMPIONONA, B., RABEFIRAISANA, H.J., JANKULOSKI, L., RAKOTONJANAHARY, X.R.T. (2018) Induced Mutation for Developing Mutant Rice Lines Tolerant to the Parasitic *Striga asiatica* (L.) Kuntze. Poster 151 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

SULIMAN, S., GHANIM, A.M.A. (2018) Mutation-Induced Variability for Improved Yield in Spring Wheat Under Hot Irrigated Environments. Poster 248 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

AL-MAMARI, A.H.K., GHANIM, A.M.A. (2018) Mutation Induction for Sorghum And Rice Using Gamma and X ray Irradiations. Poster 251 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

DANSO, K., GHANIM, A.M.A., DWARKO, D., JANKULOSKI L., NUNEKPEKU, W. (2018) Dose Optimisation for Pollen and Embryos Irradiation Mutation Induction in Oil Palm. Poster 221 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

GRAUSGRUBER, H. EMSENHUBER, C., KLEVENHUSEN, F., HOCHHAUSER, F., JANKULOSKI, L., ZEBELI, Q. (2018) Evaluation of Hooded (Kap1), Awnless (Lks1) and Orange Lemma (*Rob1*) Mutants of Barley (*Hordeum vulgare* L.) for their Use as Forage Crop. Poster 276 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

SARSU, F., GHANIM, A.M.A., PAREEK, A., FORSTER, B.P., INGELBRECHT, I., ASHRAF, M., KUSOLWA, P.M., DAS, P., BAHUNGUNA, R.N., SINGLA-PAREEK, S.L. (2018) Screening Protocols for Heat Tolerant Mutants in Rice. Poster 273 presented at the FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology, 27–31 August 2018, Vienna, Austria.

EXTRA BUDGETARY SUPPORT

A Peaceful Uses Initiative (PUI) project on 'Enhancing climate change adaptation and disease resilience in banana-coffee cropping systems in East Africa', 2019-2021, received support (350.000 Euro) from Belgium. This is a joint project between the Soil and Water Management and Crop Nutrition and the Plant Breeding and Genetics Sub-Programmes. This project aims to better understand how soil and water management and crop varieties can be improved for better climate change adaptation and enhanced disease resilience of banana-coffee cropping systems in East Africa. It will focus on the development and use of isotope and nuclear techniques and related biotechnologies to improve varieties for better yield and disease resistance in coffee and banana and enhance their climate change resilience through more efficient use of soil nutrients and water.

THE SOIL AND WATER MANAGEMENT & CROP NUTRITION LABORATORY

EXECUTIVE SUMMARY

The Soil and Water Management & Crop Nutrition Laboratory (SWMCNL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture assists Member States in the development and transfer of isotopic and nuclear technologies to improve the resilience of farmers' communities to climate change through climate-smart agriculture, including soil and water conservation and optimization of 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 2018, the SWMCNL conducted a wide range of activities: (i) it developed robust and affordable isotope, nuclear and related conventional techniques for climate-smart agriculture; (ii) supported the improvement of nuclear emergency preparedness and response in food and agriculture, (iii) trained technical staff and scientists from Member States in the use of nuclear and related techniques to assess climate change impact and develop climate-smart soil and water management practices; (iv) carried out isotope analyses for research and development (R&D); and (v) provided quality assurance services to Member States.

The R&D activities at the SWMCNL included novel applications of isotope and nuclear techniques to identify sediment pathways across arable land, with emphasis on precision identification of sediment sources. Tests were implemented to use cosmic-ray neutron sensor technology to calibrate satellite imagery for soil moisture monitoring to advise on agricultural water management. A technology package was developed on how to use laser isotope analysis to measure the carbon-13 signature of carbon dioxide for tracing greenhouse gas emissions from soil. Techniques for stable isotope labelling of plant materials were significantly improved for tracing carbon cycling in agroecosystems. Initial steps were made to develop a package of stable isotope techniques to measure water use efficiency and water stress tolerance to counteract drought effects on cassava systems. Important progress was made in nuclear emergency preparedness and response in food and agriculture, by implementing R&D on the prediction of radiocaesium accumulation in edible parts of crops. All these activities are essential in supporting the implementation of the five Coordinated Research Projects (CRP) of the SWMCN Subprogramme, two of which are coordinated by the SWMCNL, i.e. CRP D1.50.17 on *'Nuclear Techniques for Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems'* and CRP D1.50.15 on *'Response to Nuclear Emergencies Affecting food and Agriculture'*.

A major component of the work of the SWMCNL is its significant contribution to training and capacity building in Member States. The SWMCNL hosted 12 fellows and 4 interns from 7 countries, for training on the use of isotopic and nuclear techniques to assess climate change impacts on soil and water resources, improve agricultural water management and soil conservation in support of climate-smart agriculture.

An overview of 20 years of R&D work carried out by the SWMCN Subprogramme on how isotopic techniques can help combat soil degradation was published in a top ranked peer-reviewed journal. R&D Information was further communicated to Member States through 46 publications as book chapters, conference papers and publications in international peer-reviewed journals, including one book, and three TECDOCs on the use of isotope and nuclear techniques for improving agricultural water management.

The SWMCNL analysed a total of 4,801 and 300 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 optimize soil and water management practices. Emphasis was also put on ^{13}C-CO_2 and ^{15}N-N_2O measurements using the laboratory-based laser isotope analysers.

STAFF

Name	Title
Dercon, Gerd	Laboratory Head
Mabit, Lionel	Soil Scientist
Wahbi, Ammar	Soil Scientist
Chen, Janet	Associate Climate Change Impact Analyst (JPO)
Heiling, Maria	Senior Laboratory Technician
Weltin, Georg	Senior Laboratory Technician
Resch, Christian	Senior Laboratory Technician
Toloza, Arsenio	Laboratory Technician
Gruber, Roman	Laboratory Technician
Jagoditsch, Norbert	Technical Attendant
Mletzko, Joanna Malgorzata	Team Assistant
Fesenko, Sergey	Consultant
Lee Zhi Yi, Amelia	Consultant
Mayr, Leo	Consultant
Slaets, Johanna	Consultant
Merckx, Roel	Consultant
Zhang, Hanlin	Consultant
Chan, Neng-Iong	Intern
Falcao, Raquel	Intern
Van Laere, Jonas	Intern
Zhang, Jie	Intern

STAFF

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

The Soil and Water Management & 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 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 plot (on-farm) or area-wide level.

The SWMCNL also supports Member States to be better prepared in responding to 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 global food security. Changes in weather patterns, with increasing severity of storms, floods, droughts and extreme temperatures, impact sustainable agricultural production. These increasingly amplify soil erosion, land degradation and crop failures worldwide. Agriculture can further accelerate climate change due to the greenhouse gas it emits. 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-smart agriculture.

New research on compound-specific stable isotope (CSSI) techniques for the precision determination of sediment sources

Based on SWMCNL studies carried out in 2017, investigating sediment origins across small-scale cultivated fields (Mabit et al., 2018) [1], new R&D activities were initiated under CRP D1.50.17, in collaboration with the Austrian Institute for Land and Water Management Research, to apply CSSI techniques for determining the field origin of sediment transported and deposited in larger Austrian agricultural watersheds.

For this new phase of studies and tests, the experimental and instrumented watershed of Petzenkirchen (66 hectares) was selected. This watershed is located about 100 km west of Vienna and has been and still being studied for several agro-environmental purposes by different Austrian research institutions. The climatic conditions of the Petzenkirchen area can be characterised as temperate with continental influences, a mean yearly temperature of 9.5˚C and a precipitation of 823 mm. The dominant soil types are Cambisols and Planosols.

A sampling strategy such as the one applied in the 2017 study (located in Mistelbach) was selected to determine the origin of the sediment produced at the outlet of the Petzenkirchen watershed. The contributing area of the watershed to its outlet has been calculated to be ca. 50 ha considering the connectivity of the catchment and the sources that were most likely to contribute to the sediment at the outlet. Indeed, considering (i) the geomorphology of the site and the flow of the runoff, (ii) the significant interaction of roads, and (iii) the distance and connection of the sources to the outlet, some parts of the studied watershed were excluded (Figure 1).

Field work associated with the sampling strategy consisted of the collection of composite samples (sampling procedure repeated 15 to 20 times at several intervals across the sampling area of each specific source) at each potential sediment source. In total twelve agricultural sediment sources and four additional potential sources (i.e. streambank, riparian zone (wetland), pasture, forest) were identified. Finally, the sediments deposited at the outlet of the watershed were collected.

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[1] Mabit, L., Gibbs, M., Mbaye, M., Meusburger, K., Toloza, A., Resch, C., Klik, A., Swales, A., Alewell, C. (2018). Novel application of a Compound Specific Stable Isotope (CSSI) technique to investigate on-site sediment origins across arable fields. Geoderma, 316, 19–26.

All samples were prepared and homogenized at the SWMCNL and then sent to the National Institute of Water and Atmospheric Research of New Zealand for extraction of fatty acids (FAs) and determination of their δ^{13}C signature.

Based on the existing land-use records, the agricultural fields are dominated by a rotation of winter wheat (C_3 plant) followed by maize (C_4 plant) cultivation. As highlighted by Mabit et al. (2018), agricultural fields having different mid-term historical records may present significant differences in the δ^{13}C isotopic signature of their long-chain saturated fatty acids (FAs) and in some cases as well in their bulk δ^{13}C signature. Therefore, to avoid any missing source, each individual agricultural field was considered as an independent and potential source. The first analytical results showed that the long-chain FAs, C24:0 and C26:0, are the best fingerprints for identification of the sediment sources.
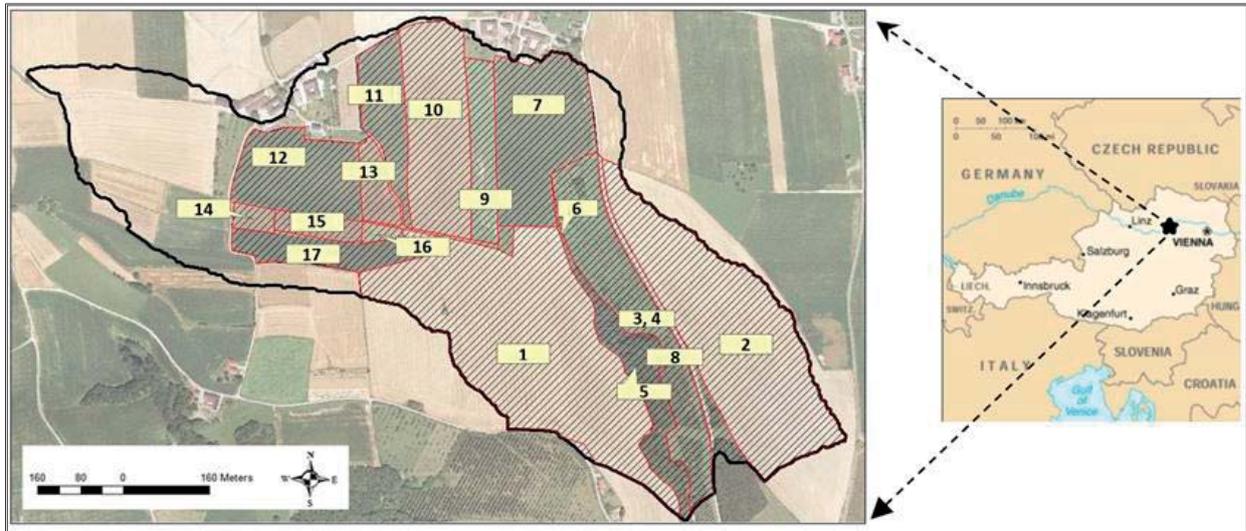

FIG. 1: *Location of the Petzenkirchen watershed and the various potential sediment sources investigated. The outlet is located at the southernmost point of the watershed.*

Exploring innovative techniques for identifying geochemical elements as fingerprints of sediment sources in an agricultural catchment of Argentina affected by soil erosion

Identification of hot spots of land degradation is strongly related to the selection of soil tracers for sediment pathways. With the support from the STEP fellowship programme[2] this study investigated the complementary and integrated application of two analytical techniques to select the most suitable fingerprint tracers for identifying the main sources of sediments in an agricultural catchment located in Central Argentina with erosive loess soils. Diffuse reflectance Fourier transformed in the mid-infrared range (DRIFT-MIR) spectroscopy and energy-dispersive X-ray fluorescence (EDXRF) were used for a suitable fingerprint selection. For using DRIFT-MIR spectroscopy as fingerprinting technique, calibration through quantitative parameters is needed to link and correlate DRIFT-MIR spectra with soil tracers.

EDXRF was used in this context for determining the concentrations of geochemical elements in soil samples. The selected tracers were confirmed using two artificial mixtures composed of known proportions of soil collected in different sites with distinctive soil uses. These fingerprint elements were used as parameters to build a predictive model with the whole set of DRIFTMIR spectra. Fingerprint elements such as phosphorus, iron, calcium, barium, and titanium were identified for obtaining a suitable reconstruction of the source proportions in the artificial mixtures. Mid-infrared

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[2] Through its Sandwich Training Educational Programme (STEP), the International Centre for Theoretical Physics and its UN partner, the International Atomic Energy Agency (IAEA), offer fellowships to PhD students from developing countries in the fields of physics and mathematics.

spectra produced successful prediction models (R^2 = 0.91) for Fe content and moderate useful prediction (R^2 = 0.72) for Ti content. For Ca, P, and Ba, the R^2 were 0.44, 0.58, and 0.59 respectively.

Technology package developed on using carbon laser isotope (^{13}C-CO$_2$) analysis for climate-smart agriculture

SWMCNL successfully developed a technology package for the use of carbon laser isotope analysis to trace carbon dioxide emissions from soil. Standard Operating Procedures (SOPs) were prepared and tested for producing ^{13}C-labelled CO$_2$ gas for calibration purposes and using the continuous basic-free flow and injection/batch modes to trace and measure carbon dioxide release.

SOPs providing step-by-step instructions on how to perform analyser measurements and data analysis with illustrations were developed. These SOPs supported by one case study have now been compiled into a TECDOC that will guide users in the operation of the carbon isotope analyser, as well as in data analysis that can ultimately be used to evaluate soil management practices. The TECDOC is expected to be available for Member States in 2019.

New developments in the homogeneous ^{13}C labelling of plant material using laser isotope analyser

To produce homogeneous ^{13}C labelled plant material, the SWMCNL developed a continuous labelling process whereby the air ^{13}C-CO$_2$ signature in a walk-in growth chamber is controlled through laser isotope analysis. Labelled plant material can be used to trace carbon cycling in agroecosystems, increasing our understanding of the effect of cropping practices on carbon sequestration and greenhouse gas emissions. Current ^{13}C labelling methods largely fall into two categories: pulse and continuous labelling. When pulse labelling, the stabile and labile fraction of the plant do not end up with the same isotopic signature, which makes quantitative tracing impossible. This issue is resolved with continuous labelling, typically done in small plexiglass chambers. The challenge here is keeping the environmental conditions, including the air isotopic signature, constant. The advent of laser spectroscopy opens the frontier to fully control the ^{13}C-CO$_2$ levels in the chamber air.

The SWMCNL has therefore pioneered the use of a laser isotope analysis to control the air ^{13}C-CO$_2$ isotopic signature in a walk-in growth chamber used for labelling relatively large amounts of biomass (up to 1 kg dry matter). The controlling of the gas mixture automatically keeps the ^{13}C signature in the CO$_2$ of the air between pre-defined threshold values, ensuring the production of high-quality homogeneous labelled material. A successful labelling with maize was conducted in 2018, and the approach of homogenous ^{13}C labelling by controlling air ^{13}C-CO$_2$ with a laser isotope analyser was proven to be valid.

Overall, the advancements will enable the SWMCNL to produce homogeneous ^{13}C labelled plant material, which can be used by Member States for increased understanding of C cycling in their agroecosystems.

Tracing carbon dioxide emissions in a soybean and maize rotation system

With the establishment of carbon laser isotope (^{13}C-CO$_2$) analysis and ^{13}C plant labelling protocols near completion, we are now conducting new R&D to track and trace carbon emissions from soils using natural differences in δ^{13}C. Plants that use C$_3$ photosynthesis have a more ^{13}C-depleted signature compared to plants that use C$_4$ photosynthesis, and this natural difference in δ^{13}C among plants can be used to determine the proportion of each plant source in different pools of carbon, such as CO$_2$ emissions from soil respiration.

An ongoing greenhouse mesocosm experiment with an annual soybean and maize rotation was used to get baseline measurements of soil respiration ^{13}CO$_2$ signatures. This was done by measuring soil respiration with the carbon isotope analyser immediately after harvest of mature soybean (C$_3$ photosynthesis) or maize shoot (C$_4$ photosynthesis) material. It was assumed that an isolated root respiration signal would be strongest immediately after harvesting the respective mature shoot

material. In the mesocosm experiment, two different soil types were used: Cambisol (poor in organic matter) and Chernozem (rich in organic matter) soils.

Keeling plot estimates of the $\delta^{13}C$ signal of the CO_2 soil respiration were, as predicted, more ^{13}C-depleted (by nearly 4‰) in mesocosms, containing Cambisol soils without crop residues, right after soybean harvest. No difference, however, was observed in mesocosms containing Chernozem soils (Table 1). The $\delta^{13}C$ signal of the CO_2 respiration from soils with crop residue application varied only in Chernozem soils and had predicted $\delta^{13}C$ CO_2 source values. These preliminary measurements will be used to estimate the contribution of below ground biomass of soybean and maize to soil respiration in soybean-maize rotation systems. A new set of analyses will be carried out in 2019 for validating the first run of experiments, especially the unexpected values for the mesocosm with Chernozem soil without crop residues.

Table 1. Mean $\delta^{13}C$ (‰) of Keeling plot source CO_2 estimates (± SE) of 3 mesocosm measurements each at time of mature maize shoot harvest and mature soybean shoot harvest in mesocosms without (-M) and with (+M) crop residue application

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	Cambisol (−M)	Cambisol (+M)	Chernozem (-M)	Chernozem (+M)
Maize harvest	-14.18 ± 2.23	-16.07 ± 2.45	-7.88 ± 11.76	-10.31 ± 0.06
Soybean harvest	-18.63 ± 0.62	-17.35 ± 2.56	-7.11 ± 2.85	-16.98 ± 3.28

Assuring the quality of ^{15}N labelling of plant material

^{15}N labelled plant material has important applications for tracing nitrogen through soil, water and air. In applications where the plant material is not fully homogenized through milling or grinding, it is important to understand the variability of the labelling for quantification of N fluxes. While the heterogeneity between plant organs (for example maize leaves, cobs and roots) is often assessed, the variation within plant leaves remains poorly understood, to date. Therefore, a pot experiment was set up in the SWMCN Laboratory to assess the variation of the ^{15}N isotopic signature resulting from enriched fertilizer within maize leaves.

In the growth chamber, maize seeds were planted in 9 pots filled with sand. The pots were continuously watered with two different ^{15}N-enriched fertilizer solutions: pure labelled nitrate, and labelled nitrate mixed with a commercial N fertilizer containing both (unlabelled) ammonium and nitrate. Three leaves were harvested per maize plant. The ^{15}N signature in each leaf was assessed near the maize stem, in the middle of the leaf and near the tip of the leaf.

Our results yield important recommendations for the use of labelled material in ^{15}N studies (Figure 2). First, it is crucial to harvest the whole plant and homogenize with extreme care when material has been labelled in the field, for example, in fertilizer use efficiency studies. Second, in experiments where coarse ^{15}N labelled plant material is used for assessing N movement (e.g. labelled mulch or compost), it is essential to develop the material using only one N-source to reliably quantify nitrogen fluxes.
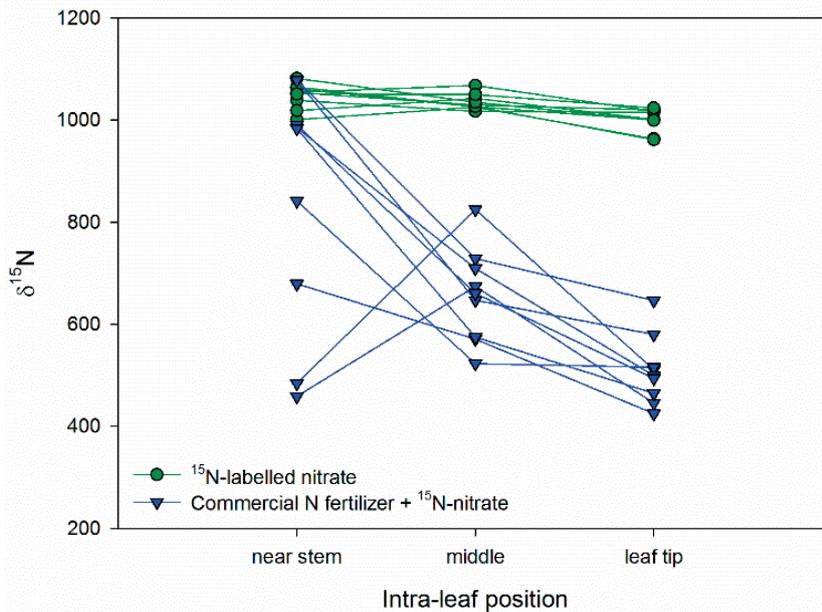
FIG. 2: *Isotopic signature variation within maize leaves. Commercial fertilizer contained both unlabelled ammonium and nitrate. Maize plants exclusively treated with nitrate did not demonstrate strong intra-leaf variation (Green circles). When using ^{15}N-labelled nitrate in the presence of unlabelled ammonium, the leaf base is enriched compared to the leaf tip (up to 600 δ ^{15}N difference from leaf base to leaf tip, blue triangles). Presence of two N-pools thus results in large gradients in ^{15}N-signature in maize leaves.*

Assessing impact of changing precipitation on nitrous oxide emission through a stable isotope and lysimeter study

In collaboration with the University of Natural Resources and Life Sciences Vienna (BOKU) and the Austrian Agency for Health and Food Safety (AGES), a method was tested at the AGES long-term lysimeter facilities to assess the impact of changing rainfall on nitrous oxide emissions from agricultural soils. For two soil types (sandy calcaric Phaeozem and calcic Chernozem), representative for the Marchfeld vegetable growing region in North-East Austria, and two rainfall regimes (current versus reduced), the mineralization cycle of nitrogen of green manure (*Sinapis alba*), dual labelled with ^{13}C and ^{15}N stable isotopes, was assessed using isotopic techniques.

Nitrous oxide (N$_2$O) and the corresponding isotopes were measured by in-situ laser isotope analysis. Highest N$_2$O emissions were observed after nitrogen fertilizer application, δ^{15}N-N$_2$O measurements indicated very low contributions to N$_2$O emissions from green manure during both studied rainfall regimes. No visible impact of reduced precipitation on N$_2$O emission could be observed.

Validation of spaceborne soil moisture products using a cosmic-ray neutron sensor (CRNS) in Petzenkirchen, Austria

In collaboration with the Vienna University of Technology (TU Wien) and the Federal Agency of Water Management (BAW), soil moisture data, collected at the Hydrological Open Air Laboratory in Petzenkirchen, Lower Austria, with a stationary CRNS since 2013, have been compared to satellite data and the network of point measurements by conventional methods, e.g. gravimetric sampling and Time Domain Transmission (TDT) measurements distributed within the CRNS footprint.

Satellite soil moisture retrievals from satellite imagery of ASCAT has a 25-km resolution, while Sentinel-1 one-km resolution. The Time Domain Transmission (TDT) sensor network measured soil moisture at different soil depths. Despite the negative bias between CRNS and the other data sets, the correlations between all data sets is relatively high. Temporal correlation as indicated by Pearson's correlation

coefficient is 0.75 between point data and ASCAT, 0.65 between TDT and CRNS, and 0.49 between CRNS and ASCAT. For Sentinel-1 correlation coefficients of 0.59 and 0.51 were found with TDT and CRNS respectively. When calculating the correlations between the soil moisture anomalies, the temporal correlation increases for ASCAT and Sentinel-1 with: 0.68 and 0.55 between TDT and ASCAT and Sentinel-1, 0.53 and 0.61 between CRNS and ASCAT and Sentinel-1 and 0.69 between CRNS and TDT.

The high anomaly correlation coefficients values reflect the fact that all soil moisture data sources capture rainfall events very well. Despite vegetation impacts the CRNS and satellite measurements the fact that in general no seasonal biases or other artefacts can be observed in both time series suggests that vegetation is treated correctly in the CRNS and satellite estimates over the Hydrological Open Air Laboratory. However, during the summer of 2016 the bias between CRNS and the other data sets, which is overall negative, decreased and higher soil moisture values were measured with CRNS (Figure 4). This was possibly caused by the presence of maize within the CRNS footprint, which has a high vegetation water content. The high correlations between CRNS and both the satellite and TDT data, for absolute values and anomalies, emphasizes the high potential of the CRNS technique for monitoring of field-scale soil moisture. Nonetheless, further research to study scaling effects and the impact of vegetation and soil properties will be carried out in the next years.
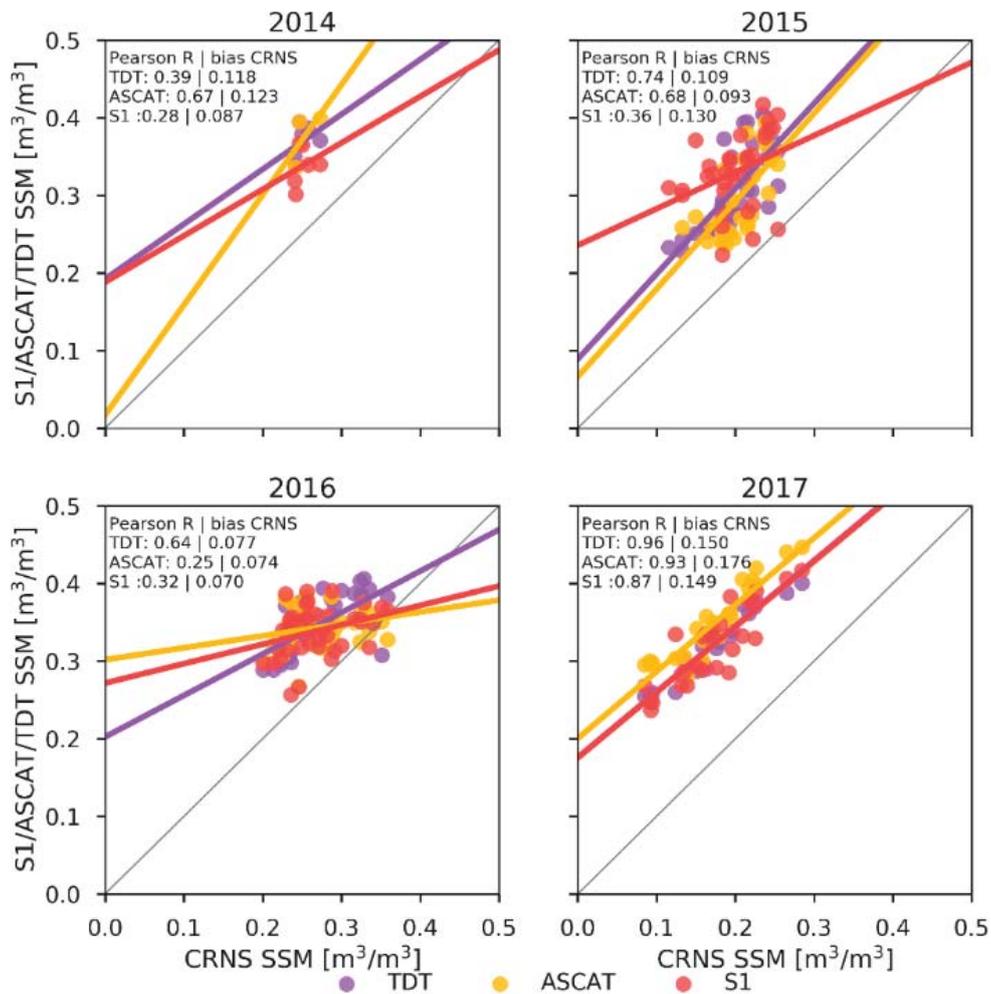

FIG. 3: *Scatterplots of CRNS, ASCAT, Sentinel-1 (S1) and TDR data on soil moisture (SSM) for every year. Temporal correlation as indicated by Pearson R and bias between CRNS and the three data sets are calculated for every year*

Footprint and effective depth of mobile cosmic-ray neutron sensor technology

To further improve the application of cosmic-ray neutron sensor technology, the SWMCNL validated the footprint, effective measurement depth and accuracy of the mobile or "backpack" version of the sensor. Through sixteen different calibrations at five research sites in Austria, located between 300 and 1,700 m a.s.l., a comparison was made between the volumetric water content measured by the backpack and gravimetric measurements for different radii of influence. Results indicated similar outcomes based on a 0-75 meter footprint as compared to a 0-200 meter study (Figure 5) confirming that measurements by the mobile cosmic-ray neutron sensor have a footprint with a 200-meter radius (i.e. 20 hectares). The same data were also used to determine the effective measurement depth (Figure 6), revealing that the effective depth is about 10 cm for volumetric water contents ranging between 30 and 60%.

In 2019, the SWMCNL will focus on improving the calibration methods for the cosmic-ray neutron sensors, by having more data points near the sensor, which could play a major role in error reduction.
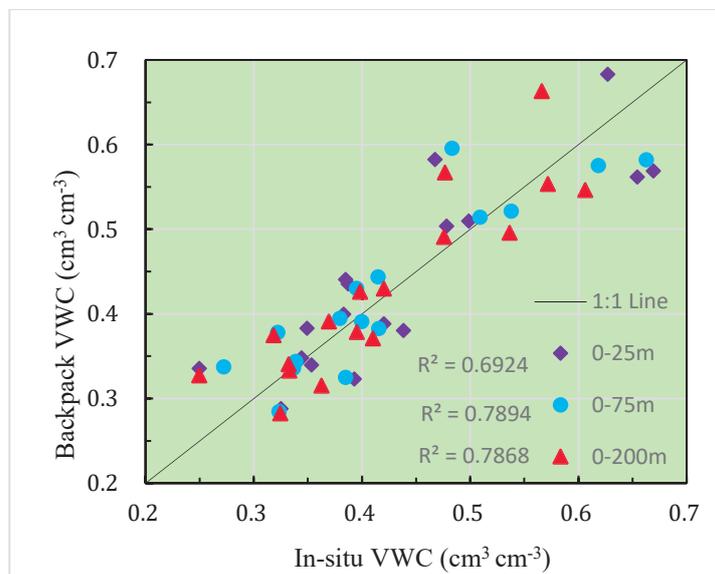

FIG. 4: Relation between in-situ and mobile cosmic ray neutron sensor (CRNS) backpack volumetric water content (VWC) for three different footprints at different elevations (from 300 to 1700 m a.s.l.) across study sites in Austria
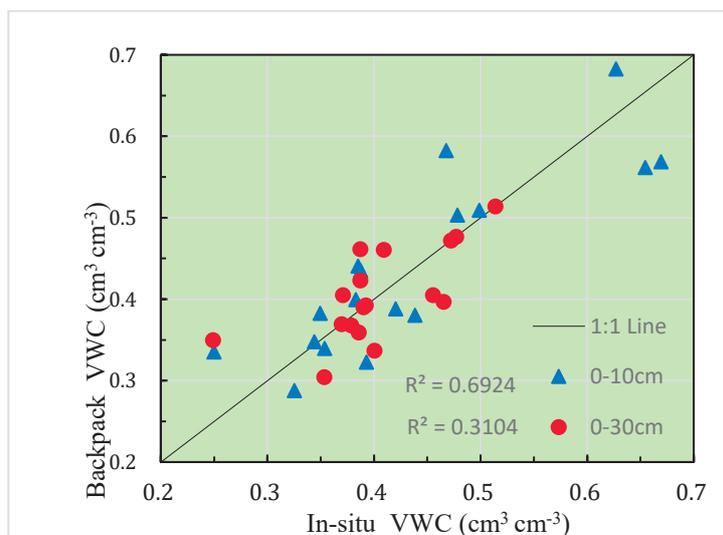

FIG. 5: Relation between in-situ and mobile cosmic ray neutron sensor (CRNS) backpack volumetric water content (VWC) for two different soil depths at different elevations (from 300 to 1700 m a.s.l.) across study sites in Austria.

Testing the efficiency of N₂O removal from nitrate containing water samples with N₂-purging for improving the bacterial denitrifier method

The bacterial denitrification method to measure the $\delta^{15}N$ and $\delta^{18}O$ signatures of nitrate containing water samples is getting more popular because of the advantages it offers such as the small sample volume, no addition of toxic chemicals, and the ability to measure even low concentrations of nitrates. In this method, bacteria are used to convert nitrates to N_2O gas which is then measured by laser isotope spectroscopy. But water samples can contain dissolved primary N_2O, which must be removed prior to bacterial conversion of nitrates, otherwise it would affect the quality of the results of the targeted nitrate isotope analysis.

One essential step of the bacterial denitrification method is purging the bacterial broth with N_2 before adding the water sample to it. Such purging also could be used to remove primary N_2O in water samples, before the bacterial conversion is carried out. To proof the efficiency of such purging, the SWMCNL conducted an experiment in which 35 ml N_2O gas with known $\delta^{15}N$ isotope signature was injected into distilled water and into a nitrate solution. Four different purging times were applied, i.e. ½ hour, 1 hour, 2 hours and 3 hours. The preliminary results showed that all tested purging times were sufficient to reduce the N_2O concentrations but the isotopic signature of $\delta^{15}N$ of N_2O proved that only 2 and 3 hours purging led to complete removal of N_2O. Two hours purging could be an effective and cheap way to remove N_2O in water samples. In the future, real field samples will be collected to test the efficiency of purging.

Cassava productivity and stable isotope methods: CIALCA takes off in Seibersdorf

Cassava (*Manihot esculenta Crantz*) has for a long time solely been a staple crop but has gained interest from industry more recently. The industry needs a year-round supply of fresh cassava roots and therefore contracts farmers to spread the planting times. This force plants to grow during dry spells, with lower yields, consequently. To counteract drought effects in cassava production, a collaboration was established between the International Institute of Tropical Agriculture (IITA) and the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, through the CIALCA project (Consortium for Improving Agriculture-based Livelihoods in Central Africa, www.cialca.org), funded by the Government of Belgium from 2018 until 2020. The main objective of this collaboration is to develop packages of stable isotope and related techniques for guiding efforts towards increasing water use efficiency (WUE) of cassava-based production systems in Central Africa.

In the first year of the project, the SWMCNL initiated the development of standard sample preparation and measuring procedures for ^{13}C and ^{18}O, indicators for WUE and stomatal conductance respectively.

Exploratory tests were made, to find the optimal method to extract cellulose from large numbers of cassava leaves or other plant organs. Reasons to go for cellulose extraction build on literature, stating that the stable isotope composition of cellulose is more appropriate than that of the bulk material, as (i) the latter is of a more variable composition including species and growth phase dependent components, (ii) the $^{18}O/^{16}O$ ratio in cellulose reflects the one of plant water at the time of cellulose synthesis and is from then onwards no longer exchangeable with its environment and hence a more reliable indicator of stomatal conductance (g_s) and (iii) the $^{13}C/^{12}C$ ratio in cellulose also seems less influenced by the same and accordingly a better indicator for WUE.

These initial try-outs were performed on shoot samples from two cassava varieties obtained through IITA, from their large number of field trials within the framework of ACAI (African Cassava Agronomy Initiative) a Bill and Melinda Gates Foundation sponsored project. The chosen method was based on NaClO₂ bleaching and followed by NaOH removal of beta- and gamma-celluloses according to Leavitt

and Danzer (1993)[3]. Cellulose samples were subject to $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratio analysis at SWMCNL and KU Leuven, respectively.

The first results showed ^{13}C and ^{18}O isotope values of extracted cellulose are in line with literature (Figure 6). They also confirmed that (i) cellulose can be extracted from cassava leaves and lead to isotope ratios that are very reproducible with little variation, and that (ii) the longer the bleaching time, the more negative the $\delta^{13}C$-values and the larger the $\delta^{18}O$-values. This points to a more effective lignin removal, as lignin is reported to have higher $\delta^{13}C$-values and lower $\delta^{18}O$-values. However, the relationship between the ^{13}C and ^{18}O values of the bulk shoot material and cellulose extract differed for both investigated varieties, with a constant difference between these values for Kiroba variety and a decreasing difference with higher water stress levels (higher ^{13}C-values) for the Mkombozi variety. This observation, in particular for ^{18}O, may be linked to the ^{18}O isotope signature of available soil water, depending on overall drought conditions. Further research is now being carried out to clarify the observed patterns, so that standard operation procedures for sampling, analysis and interpretation can be finalised.
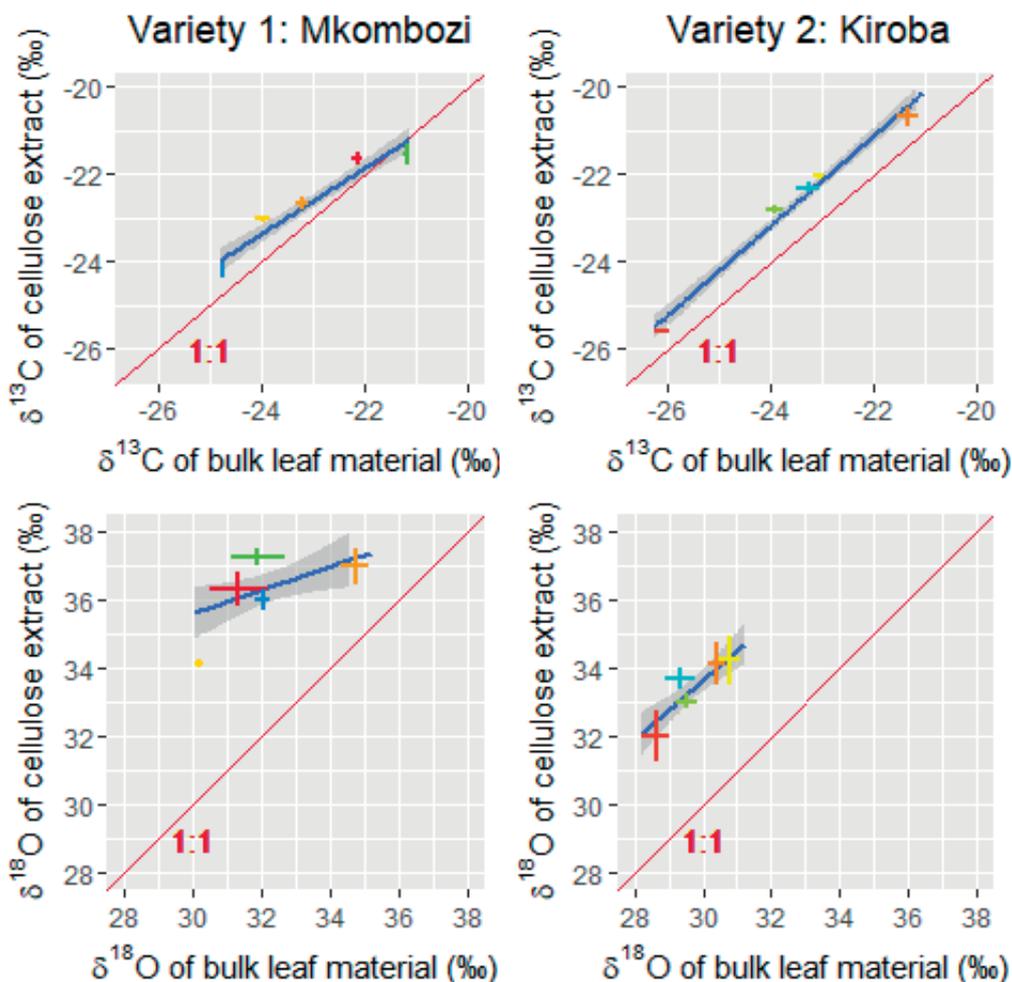

FIG. 6: *^{13}C and ^{18}O signatures of two varieties, Mkombozi (n= 5, with 2 to 4 replicates per plant sample) and Kiroba (n = 5, with 4 replicates per plant sample) from Tanzania. The red line is the 1:1 line. The grey shade represents 95% confidence interval of linear regression line. Data points, including standard deviation for both X and Y axes, with the same colour (per variety) represent one plant sample.*

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[3] Leavitt, S.W. and Danzer, S.R. (1993). Method for batch processing small wood samples to holocellulose for stable-carbon isotope analysis. Analytical Chemistry 65, 87-88.

In combination with the establishment of field trials in the CIALCA project region (Democratic Republic of Congo, Burundi and Rwanda), steps were also taken at the SWMCNL to obtain information on how $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios vary in cassava plants under the influence of water availability, for different cassava varieties. Ten cassava varieties were obtained from Burkina Faso and two from the Democratic Republic of Congo; were planted in pots of different sizes (up to 25 kg pots) in the SWMCNL greenhouse, serving as test material to explore variability in growth patterns and isotope signals. These varieties were either landraces with poor resistance to diseases and water stress or improved varieties, resistant to CMD (cassava mosaic virus disease) and moderately to very drought tolerant. Isotope analyses are now being carried out.

Some plants were transferred to the growth chamber for future development of new stable isotope techniques and indicators to assess drought tolerance and water use efficiency based on (i) C translocation speed from shoot to root (pulse ^{13}C labelling) and (ii) $^{13}C – CO_2$ signature during plant respiration (mirroring information stored in plant biomass). A first ^{13}C-CO_2 pulse labelling experiment with a calculated enrichment in the atmosphere of 43 836 ‰ was carried out and plants were harvested at multiple times, to trace the flow of assimilates from shoot to roots and how this varies with variety and time. The first results indicate that approximately 95% of the label was taken up by the plants. It is expected that the results will give more information on translocation speed.

Nuclear Emergency Preparedness in Food and Agriculture

Member States are increasingly interested in improving the capacity to respond to nuclear emergencies affecting food and agriculture due to the growing number of nuclear power plants built. Lessons learned from the Chernobyl and Fukushima Daiichi Nuclear Power Plant accidents identified critical areas for improvement and this includes data collection (sampling and analysis), data management, data visualization to make decisions swiftly, allowing food control and health authorities to respond and disseminate information to all relevant stakeholders appropriately.

Prediction of Radiocaesium Accumulation in Edible Parts of Crops

In 2018, the SWMCN Laboratory collaborated closely with the National Agriculture and Food Research Organization (NARO) of Japan, as part of the practical arrangement between NARO and the Joint FAO/IAEA Division, signed in 2016. A one-month scientific visit of one SWMCNL team member marked the first step in synergistic research supported by both institutions.

The main research theme of the exchange, initiated in preparation of a new CRP (as a follow up to CRP D1.50.15 on "Response to Nuclear Emergencies Affecting food and Agriculture"), focused on optimization of remediation of radioactive contamination in agriculture. Collaborative research was performed between NARO and SWMCNL to investigate the possibility of using information on radiocaesium (RC) accumulation in crops, such as rice and soybean, at different stages of development to predict RC accumulation in edible parts at maturity. Such information may help reduce uncertainty about the level of radiocaesium contamination at harvest. Key tasks conducted during the scientific visit were: (1) sampling and analysis of target crops, (2) data interpretation and prediction model development and (3) drafting of guidelines on the methodologies utilized in sampling.

Prior to the scientific visit, three sampling campaigns were performed by NARO for soybean and rice samples harvested at different growth stages. The SWMCNL supported in the final sampling campaign for fully matured soybean and rice plants in two field sites located in Ryozen town of Date city. Processing of plant and soil samples included drying, separating, cleaning, cutting, grinding, and placement in specialized containers for gamma spectrometry measurements. Approximately 70 soil and plant samples were collected and prepared under the guidance of NARO staff.

Rice was sampled as inedible biomass (leaf blade, sheath, and culm) and edible biomass (brown rice) at four stages – young, heading, yellow ripe and full ripe. Initial analysis of data from the rice study (n=18) showed a larger variability in measured RC concentration values for inedible parts (young (62±1 Bq/kg), heading (139±2 Bq/kg), yellow ripe (142±2 Bq/kg) and full ripe (122±3 Bq/kg)), compared to

mature edible grains that had a lower variability (yellow ripe (62±1 Bq/kg) and full ripe (51±1 Bq/kg)). Average RC concentration of inedible biomass at the heading stage was found to be nearly twice that of the young stage, but the trend stabilized during the heading, yellow ripe, and full ripe stage (Figure 7). In examining the relationship between inedible and edible parts of the rice plant at yellow ripe and full ripe stage, a clear relationship between biomass and grain during the yellow ripe and full ripe stage was found, with the K_d at harvest (K_d = edible biomass RC/inedible biomass RC) to be 0.46 (Figure 8). The cursory conclusion from the findings above is that the RC concentration in leaves stabilizes during the heading stage and can be used to predict the RC concentration in grains at maturity using the K_d at harvest. Further research is needed to link RC concentrations in young plants (inedible part) and the edible part of rice.
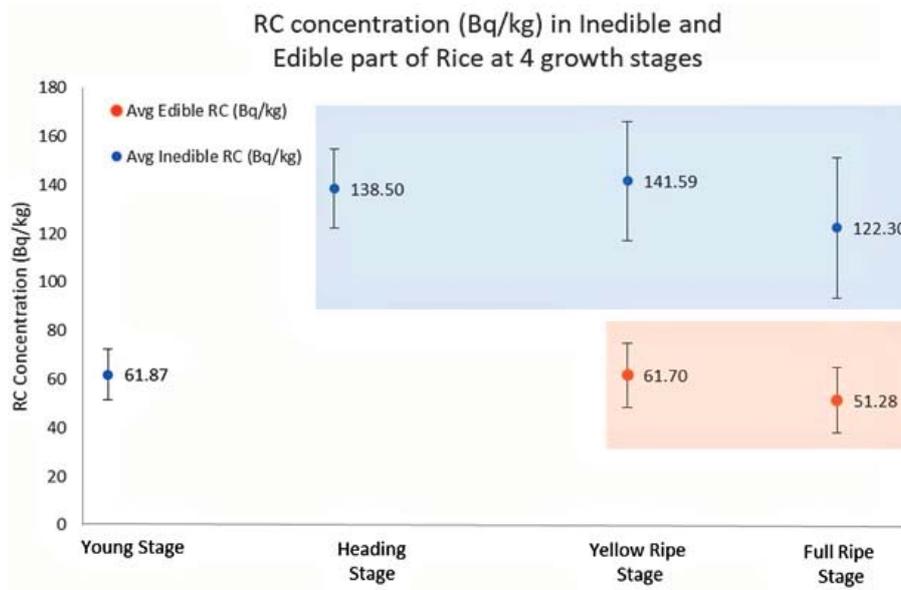

FIG. 7: *Inedible (leaf blade, sheath, and culm) and edible biomass (brown rice) in 4 stages – young (30 DAP), heading (60 DAP), yellow ripe (90 DAP) and full ripe were collected. Variability in measurements was shown to be higher in the inedible samples than edible samples, and the trend of Radiocaesium (RC) concentration in inedible parts show a general stabilization from the heading stage onwards. RC concentration nearly doubles in the inedible parts from young to heading stage.*
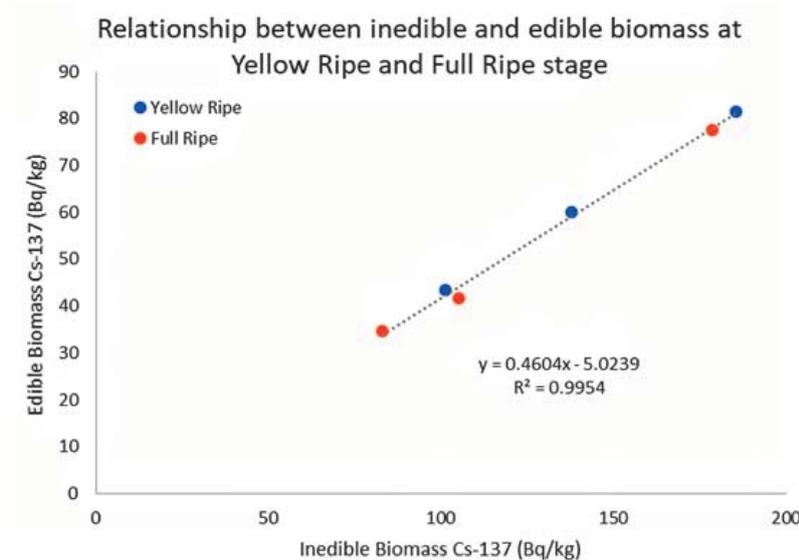

FIG. 8: *RC concentration in edible and inedible parts of the rice plant during yellow ripe stage and full ripe stage showed a good correlation and may be used as in indicator of RC concentration in edible part at harvest.*

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CAPACITY BUILDING

Regional training course on 'Methods for Assessing Impacts of Climate Change on Soil and Water Resources in Polar and Mountainous Regions', 25 June – 6 July 2018, Seibersdorf and Rauris, Austria.

In collaboration with the University of Vienna's Department of Microbiology and Ecosystem Science and the Department of Geography and Regional Science at Graz University, the SWMCNL hosted the above training course under the project INT5153 'Assessing the Impact of Climate Change and its Effects on Soil and Water Resources in Polar and Mountainous Regions'. In this project, soil and water resources in eleven benchmark sites on five continents were characterized. These very same protocols were taught to the researchers in the training course, enabling them to contribute to the established global monitoring network. To provide hands-on training in field data collection and landscape analysis, the course included a four-day field excursion to the Austrian Alps, to the municipality of Rauris and Austria's largest glacier, the Pasterze. In total, twelve scientists from seven Member States attended the course. More information on the course can be found at https://www.iaea.org/newscenter/news/bridging-the-gap-between-science-and-policy-iaea-provides-the-necessary-tools-to-climate-change-researchers .

Regional training course on 'Use of Advanced Nuclear and Related tools for Agricultural Water Management', 4-15 November 2018, Kuwait Institute of Scientific Research (KISR), Kuwait.

The SWMCN Laboratory supported a regional training course at the Kuwait Institute of Scientific Research (KISR) on the use of advanced nuclear and related tools for agricultural water management. This course was carried out under KUW5004 'Improving Production and Water Use Efficiency of Forage Crops with Nuclear Techniques'. In total 15 fellows from 5 Member States attended the course.

ANALYTICAL SERVICES

Laboratory analyses

In 2018, 4,801 samples were analysed for stable isotopes and 300 samples were measured for fallout radionuclides in the SWMCN Laboratory. Most analyses (i.e. 94%) were carried out in support of R&D at the SWMCNL focusing on the design of affordable isotope and nuclear techniques to improve soil and water management in climate-smart agriculture. An additional analytical focus of the SWMCN Laboratory was on ^{13}C-CO_2 and ^{15}N-N_2O measurements using the laboratory-based laser isotope analysers.

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 measurements provides confidence in the analytical performance of stable isotope laboratories and hence making it an important tool for external quality control. The 2018 Proficiency Test (PT) on ^{15}N and ^{13}C isotopic abundance in plant materials, organized by the University of Wageningen, the Netherlands, and funded by the SWMCN Laboratory was successfully completed. The Wageningen Evaluating Programs for Analytical Laboratories (WEPAL, http://www.wepal.nl) is accredited for the organization of Inter-Laboratory Studies by the Dutch Accreditation Council.

Every year, one ^{15}N-enriched plant test sample is included in one round of the WEPAL IPE (International Plant-Analytical Exchange) Programme. A special evaluation report for FAO/IAEA participants on the analytical performance in stable isotope analysis is issued by the SWMCN Laboratory and sent to the participants together with a certificate of participation, along with the regular WEPAL evaluation report. The participation fee for one round per year is covered by the IAEA.

In total, eleven stable isotope laboratories participated in the PT-round 2018: Africa (1): Morocco; Asia and the Pacific (3): New Zealand, Pakistan and Philippines; Europe (4): Austria, Belgium, Germany and France; Latin America (3): Argentina, Brazil and Chile. All nine laboratories participating in the nitrogen analysis test reported ^{15}N-data within the control limits for the enriched plant sample and seven out of nine participating laboratories in carbon analysis test reported ^{13}C isotopic abundance results within the control limits.

GUIDELINES AND INFORMATION PUBLISHED IN 2018

New open-access FAO/IAEA publication: Cosmic Ray Neutron Sensing: Estimation of Agricultural Crop Biomass Water Equivalent

This open access book provides methods for the estimation of Biomass Water Equivalent (BEW), an essential step for improving the accuracy of area-wide soil moisture by cosmic-ray neutron sensors (CRNS). Three techniques are explained in detail: (i) traditional in-situ destructive sampling, (ii) satellite based remote sensing of plant surfaces, and (iii) biomass estimation via the use of the CRNS. The advantages and disadvantages of each method are discussed along with step by step instructions on proper procedures and implementation. More information on the open-access publication can be found at: https://link.springer.com/book/10.1007%2F978-3-319-69539-6

Soil Moisture Mapping with a Portable Cosmic Ray Neutron Sensor (IAEA-TECDOC 1845)

This publication is an informational guide for soil moisture mapping at landscape level through a portable 'backpack' cosmic-ray neutron sensor. This recently developed device monitors soil water content in a non-invasive way using background neutron counts. It is used to measure water content in the topsoil over wide areas, covering approximately 20 hectares with one single measurement. Through its mobility and combining series of measurements, it provides the spatial variability of the soil water content for better agricultural water management. The publication gives scientists, technicians and students the necessary information, guidance and steps to calibrate, validate and deploy the portable cosmic-ray neutron sensor. It is related to CRP D1.50.17 on 'Nuclear Techniques for Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems' and CRP D1.20.13 on 'Landscape Salinity and Water Management for Improving Agricultural Productivity'. The publication can be found at: https://www-pub.iaea.org/books/IAEABooks/12357/Soil-Moisture-Mapping-with-a-Portable-Cosmic-Ray-Neutron-Sensor

Sampling and Isotope Analysis of Agricultural Pollutants in Water (IAEA-TECDOC 1850)

Stable isotope techniques can help identify the sources of water pollution associated with agricultural activities. Knowing the origin of nutrients or contaminants is essential to improve agricultural practices. To ensure the quality of stable isotope analysis, appropriate sampling and sample preparation are crucial. This publication presents methods for surface water sampling and sample processing through micro-diffusion and bacterial denitrification combined with laser spectroscopy. Information on such methods is often described in a very summarized and non-comprehensive way without proper illustration of every step. This publication aims to bridge this gap for scientists, technicians and students. It presents a selection of standard operating procedures, providing guidance in water sampling and sample preparation that are mandatory when conducting reliable isotope analysis on water. This publication is related to CRP D1.50.18 on 'Multiple Isotope Fingerprints to Identify Sources and Transport of Agro-contaminants'. This publication can be found at: https://www-pub.iaea.org/books/iaeabooks/12374/Sampling-and-Isotope-Analysis-of-Agricultural-Pollutants-in-Water

Challenges and Opportunities for Crop Production in Dry and Saline Environments in ARASIA Member States (IAEA-TECDOC 1841)

This publication serves as a reference guide for Member States and interested specialized readers wishing to work on agriculture in dry and saline environment, in particular, those located in the Middle East region. All information and instructions given in this guide are based on successful and sound practices applied in pertaining Member States for sustainable cropping of salt affected soils. It will help scientists and farmers to select management alternatives most efficient for agriculture in saline environments within their own countries. The publication also focuses on the possible use of isotopes techniques in dealing with salinity and drought conditions affecting crop production. The publication can be downloaded via https://www-pub.iaea.org/books/iaeabooks/12305/Challenges-and-Opportunities-for-Crop-Production-in-Dry-and-Saline-Environments-in-ARASIA-Member-States

Open-access publication in top ranked scientific journal reporting 20 years of soil erosion activities at the SWMCN Subprogramme

An overview of 20 years of R&D work carried out by the SWMCN Subprogramme on how isotope and nuclear techniques can help combat soil degradation was published in the top ranked peer-reviewed journal ''Land Degradation & Development'' (Mabit et al. 2018 a). It highlights the progress made within CRP D1.50.17. The paper is available through open-access, at: https://doi.org/10.1002/ldr.3016

Sharing our research progress and connecting with international researchers through the European Geosciences Union General Assembly 2018, Vienna, Austria

About 15,000 scientists from 106 countries came together at the European Geosciences Union (EGU) 2018 General Assembly held in Vienna, Austria on 8-13 April. The SWMCN Subprogramme's activities were reported in 18 oral, poster and PICO presentations covering topics such as climate change impact analysis, new radionuclide tracers for soil erosion investigations, carbon and nitrogen cycling, area-wide soil moisture screening, and decision-making software for nuclear emergencies.

The SWMCNL, under INT5153 'Assessing the Impact of Climate Change and its Effects on Soil and Water Resources in Polar and Mountainous Regions', also hosted one EGU session titled 'Soil, water and sediment tracing for unravelling climate change dynamics in proglacial areas' which had 6 oral and 21 poster presentations from experts and counterparts involved in the project.

The links to all contributions from the SWMCN Subprogramme can be found in this annual report under the publication list at the end of the SWMCNL contribution.

Bridging the gap between science and decision making – Sharing advancements in Nuclear Emergency Preparedness tools with the EGU community

Since 2014, the SWMCN Laboratory has been developing DSS4NAFA, an IT-based decision-support system for optimizing response to nuclear emergencies affecting food and agriculture. This work, now in its final stages for beta release to the Member States, was presented to the public for the first time at the European Geophysical Union (EGU) General Assembly. Presentation of the DSS4NAFA system generated strong interest and positive responses, and the SWMCNL was invited to interview on both the EGU Geopolicy and EGU Natural Hazards online blog, open to all 15,000 members of EGU. The interviews can be found at the following links: (i) https://blogs.egu.eu/geolog/2018/09/13/geopolicy-bridging-the-gap-between-science-and-decision-makers-a-new-tool-for-nuclear-emergencies-affecting-food-and-agriculture/, and (ii) https://blogs.egu.eu/divisions/nh/2018/08/27/iaea-interview/#more-490

Contribution to the IAEA Nuclear Technology Review 2018

The SWMCNL contributed to the IAEA's Nuclear Technology Review 2018 (see pp. 29-31) by reporting on the R&D progress in the field of Nuclear Emergency Preparedness for food and agriculture. Identification of contaminated food production areas and prevention of affected products from consumption are some of the main challenges during large-scale nuclear emergencies due to the sheer amount of data that need to be processed for decision making. Management of data with IT-Decision

support systems can alleviate the logistical burden placed on decision makers for tasks such as sampler allocation and restriction actions. The Decision Support System for Nuclear Emergencies Affecting Food and Agriculture (DSS4NAFA) is a robust IT-system developed to collect and centralize information, to visualize real-time data, as well as to suggest decision actions based on pre-determined limits. It supports effective management and analysis of these large and often diverse datasets, improving the response abilities of health authorities to keep food and agriculture safe during nuclear emergencies. For more information, see: https://www-legacy.iaea.org/About/Policy/GC/GC61/GC61InfDocuments/English/gc61inf-4_en.pdf

SWMCNL success stories in 2018

Twenty-seven success stories were published by the SWMCN Subprogramme highlighting examples of country impacts derived through improving soil and water management and crop nutrition across Africa, Asia and Latin America. Two stories were prepared with the support of the SWMCNL, i.e. (i) Return to traditional terracing improves farm production in Madagascar, in: *In action - Nuclear applications in agriculture - On-the-ground success (Part IV)* and at IAEA website, and (ii) Using cosmic rays to measure moisture levels in soil in: *IAEA Bulletin on "Nuclear Technology for Climate"*. These stories can be downloaded at:

- http://www-naweb.iaea.org/nafa/resources-nafa/IAEAsuccessIV_stories_Rev6.pdf
- https://www.iaea.org/newscenter/news/return-to-traditional-terracing-improves-farm-production-in-madagascar
- https://www.iaea.org/sites/default/files/publications/magazines/bulletin/bull59-3/5931617.pdf
- https://www.iaea.org/newscenter/news/using-cosmic-rays-to-measure-moisture-levels-in-soil

2018 Long Night of Research, Vienna, Austria

On 13th April 2018, about 1,600 visitors participated in the "Long Night of Research" at the IAEA Headquarters in Vienna. This event, initiated by the Austrian government, is intended to promote interest in science amongst all age groups, with special attention to children. The Agency presented 14 booths showcasing various applications of nuclear technology in the Vienna International Center. The SWMCNL prepared three exhibits on the use of stable isotope techniques in agriculture. Visitors were given the opportunity to learn about tracing pathways of agricultural pollution from various sources, efficient water and nutrient management, as well as the importance of healthy soils for crops and water quality.

United Nations Youth Forum on Sustainable Development Goals: the episode for Chinese television show "Keep Running", Vienna, Austria

This year the popular Chinese youth reality TV show "Keep Running" filmed an episode on the 17 Sustainable Development Goals of the United Nations in Vienna. In a feature segment filmed on 6th March, seven famous Chinese celebrities as well as United Nations employees were invited to speak. One of our members from the SWMCNL was invited to discuss the Joint FAO/IAEA Programme's contribution to Sustainable Development Goals 1 and 2 on no poverty and zero hunger. Success stories from Sudan and Bangladesh were used as examples of how nuclear techniques are supporting farmers to decrease water use and increase crop yield.

PUBLICATIONS

AL-MENAIE, H., AL-RAGOM, O., AL-SHATTI, A., BABU, M.A., WAHBI, A. (2018). Kuwait. In: *Challenges and Opportunities for Crop Production in Dry and Saline Environments in ARASIA Member States.* pp. 42–46. IAEA-TECDOC-1841. IAEA publication.

AVERY, W., FRANZ, T., WAHBI, A., DERCON, G., HENG, L., STRAUSS, P. (2018). Mobile Soil Moisture Sensing in High Elevations: Applications of the Cosmic Ray Neutron Sensor Technique in

Heterogeneous Terrain. In: Geophysical Research Abstracts, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018-9781. https://meetingorganizer.copernicus.org/EGU2018/EGU2018-9781.pdf.

BLAKE, W., BOECKX, P., STOCK, B., SMITH, H.G., BODE, S., UPADAHAYAY, H.R., GASPAR, L., GODDARD, R., LENNARD, A., LIZAGA, I., LOBB, D., OWENS, P.N., PETTICREW, E., KUZYK, Z.Z., GARI, B., MUNISHI, L., MTEI, K., MUNISHI, L., NEBIYU, A., MABIT, L., NAVAS, A., SEMMENS, B., LIZAGA, I. (2018). A deconvolutional Bayesian mixing model approach for river basin sediment source apportionment. *Scientific Reports*, 8:13073.

BRANDT, C., DERCON, G., CADISCH, G., NGUYEN, L.T., SCHULLER, P., LINARES, C.B., SANTANA, A.C., GOLOSOV, V., BENMANSOUR, M., AMENZOU, N., XINBAO, Z., RASCHE, F. (2018). Towards global applicability? Erosion source discrimination across catchments using compound-specific $\delta^{13}C$ isotopes. *Agriculture, Ecosystems & Environment*, 256, 114-122.

BRAVO-LINARES, C., SCHULLER, P., CASTILLO, A., OVANDO-FUENTEALBA, L., MUÑOZ-ARCOS, E., ALARCÓN, O., DE LOS SANTOSVILLALOBOS, S., CARDOSO, R., MUNIZ, M., DOS ANJOS, R.M., BUSTAMANTE-ORTEGA, R., DERCON, G. (2018). First use of a compound-specific stable isotope (CSSI) technique to trace sediment transport in upland forest catchments of Chile. *Science of The Total Environment*, 618, 1114–1124.

CASTILLO, A., GOLOSOV, V., MAVLYUDOV, B., EVANGELISTA, H., DERCON, G., NAVAS, A., SCHULLER, P. (2018). Use of fallout radionuclides and stable elements to characterize the contribution of the different sediment sources in small river catchment, in King George Island, Maritime Antarctica. In: Geophysical Research Abstracts, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018-10330. http://meetingorganizer.copernicus.org/EGU2018/10330.pdf .

CHEN, J., HEILING, M., GRUBER, R., RESCH, C., DERCON, G. (2018). $^{13}CO_2$ pulse labeled maize crop residues underestimate contribution to respiration of fresh and rejuvenated soils. In: *Geophysical Research Abstracts*, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018-6079. http://meetingorganizer.copernicus.org/EGU2018/ 2018-6079.pdf .

CHEN, J., HEILING, M., RESCH, C., M'BAYE, M., GRUBER, R., DERCON, G. (2018). Does maize and legume mulch matter in soil organic carbon sequestration? *Agriculture, Ecosystems & Environment*, 265, 123–131.

DERCON, G., EVANGELISTA, H., CASTILLO, A., GOLOSOV, V., MAVLYUDOV, B., MUSTAFA, O., MAERCKER, J., VERKULICH, S., SLAETS, J., STOTT, T. AND NAVAS A. (2018). Modern sediment redistribution in pro-glacial areas of King George Island, South Shetland Islands. In: Geophysical Research Abstracts, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018-3928, http://meetingorganizer.copernicus.org/EGU2018/3928.pdf.

EVRARD, O., MEUSBURGER, K., VAN OOST, K., BORELLI, P., KETTERER, M., MABIT, L., LEFÈVRE, I., ALEWELL, C., PANAGOS, P. (2018). Discriminating the contribution of global fallout and Chernobyl to anthropogenic fallout radionuclide inventories in soils of Europe. Session T: Radioecology and environmental studies in support of Fukushima. American Nuclear Society / International Topical Conference. Eleventh International Conference on Methods and Applications of Radioanalytical Chemistry (MARC XI), 8-13 April 2018, Kailua-Kona, Hawaii (HI), USA. Oral presentation. http://www.marcconference.org/wp-content/uploads/marcxi_BookofAbstracts_20180410.pdf.

FALCAO, R., MABIT, L., TOLOZA, A., LEE ZHI YI, A., BENMANSOUR, M. (2018). Potential application of $^{239+240}Pu$ in Austria as a soil erosion rates proxy. Divisional Symposium – Division 1. C1.6.1. Human-environment interactions recorded in soils and palaeosols. RIO18, 21st World Congress of Soil Science, 12-17 August 2018, Rio de Janeiro, Brazil. Oral presentation.

FRANZ, T.E., AVERY, W., DESILETS, D., WAHBI, A. (2018). Data processing of survey data. In: *Soil Moisture Mapping with a Portable Cosmic Ray Neutron Sensor.* pp 14–24. IAEA-TECDOC-1845. IAEA publication.

FRANZ, T.E., DESILETS, D., WAHBI, A., HENG, L., DERCON, G., AVERY, W., STRAUSS, P. (2018). Description of software and field examples of CRNS surveys. In: *Soil Moisture Mapping with a Portable Cosmic Ray Neutron Sensor.* pp 25–42. IAEA-TECDOC-1845. IAEA publication.

FRANZ, T.E., WAHBI, A., AVERY, W. (2018). Estimation of Biomass Water Equivalent via the Cosmic Ray Neutron Sensor. In: Cosmic Ray Neutron Sensing: Estimation of Agricultural Crop Biomass Water Equivalent, 25-32 (Eds Wahbi, A., Heng, L., and Dercon, G.), Springer (Open source), https://link.springer.com/chapter/10.1007/978-3-319-69539-6_4.

GREGORY, P.J., ISMAIL, S., RAZAQ, I.B., WAHBI, A. (2018). Soil salinity: current status and in-depth analysis for sustainable use. In: *Challenges and Opportunities for Crop Production in Dry and Saline Environments in ARASIA Member States.* pp 4–11. IAEA-TECDOC-1841. IAEA publication.

GRIEPENTROG, M., BODÉ, S., BOUDIN, M., DERCON, G., DOETTERL, S., EGLINTON, T., HAGHIPOUR, N., JOYA, S., MARTIN, V., MATULANYA, M., MSIGWA, A., SUN, X., VERMEIR, P., WANG, X., RICHTER, A., BOECKX, P. (2018). Climatic versus geochemical controls on soil organic matter stabilization and greenhouse gas emissions along altitudinal transects in different mountain regions. In: Geophysical Research Abstracts, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018- 12981. http://meetingorganizer.copernicus.org/EGU2018/ 12981.pdf.

HEILING, M. (2018). Micro-diffusion of ^{15}N enriched ammonium and nitrate samples. In: *Sampling and Isotope Analysis of Agricultural Pollutants in Water.* pp. 50-58. IAEA-TECDOC-1850. IAEA publication.

JANAT, M., KURDALI, F., MUBARAK, I., WAHBI, A. (2018). Syrian Arab Republic. In: *Challenges and Opportunities for Crop Production in Dry and Saline Environments in ARASIA Member States.* pp 89–97. IAEA-TECDOC-1841. IAEA publication.

KHODADADI, M., MABIT, L., ZAMAN, M., PORTO, P., GORJI, M. (2018). Using ^{137}Cs and ^{210}Pb$_{ex}$ measurements to explore the effectiveness of soil conservation measures in semi-arid lands: a case study in the Kouhin region of Iran. *Journal of Soils and Sediments*. https://doi.org/10.1007/s11368-018-2205-y.

LEE ZHI YI, A., ALBINET, F., ADJIGOGOV, L., DERCON, G. (2018). DSS4NAFA – a tool for data management, visualization and risk communication in emergencies affecting food and agriculture. In: Geophysical Research Abstracts, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018-3416. https://meetingorganizer.copernicus.org/EGU2018/EGU2018-3416.pdf.

LEE ZHI YI, A., TOLOZA, A., MABIT, L. (2018). Fine Increment Soil Collector (FISC) for expediting and standardizing sampling processes during in-situ investigations. In: Geophysical Research Abstracts, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018-5110. https://meetingorganizer.copernicus.org/EGU2018/EGU2018-5110.pdf.

LEE ZHI YI, A., YAN, T.Z., HEILING, M., RESCH, C., TOLOZA, A., WELTIN, G. (2018). ^{15}N and ^{18}O isotopic analysis of nitrate using laser spectroscopy and a bacterial denitrifier method. In: *Sampling and Isotope Analysis of Agricultural Pollutants in Water.* pp. 22-49. IAEA-TECDOC-1850. IAEA publication.

LIZAGA, I., GASPAR, L., QUIJANO, L., DERCON, G., NAVAS, A. (2018). NDVI, ^{137}Cs and nutrients for tracking soil and vegetation development on glacial landforms in the Lake Parón Catchment (Cordillera Blanca, Perú). *Science of The Total Environment*. https://doi.org/10.1016/j.scitotenv.2018.09.075.

MABIT, L., BERNARD, C., LEE ZHI YI, A., FULAJTAR, L., DERCON, G., ZAMAN, M., TOLOZA, A., HENG, L. (2018 a). Promoting the use of isotopic techniques to combat soil erosion: an overview of the key role played by the SWMCN Subprogramme of the Joint FAO/IAEA Division over the last 20 years. *Land Degradation & Development*, 29, 3077–3091.

MABIT, L., FALCAO, R., TOLOZA, A., LEE ZHI YI, A., BENMANSOUR, M. (2018 b). Can we use Pu-(239+240) isotopes as soil redistribution tracers in Austrian agro-environments? An initial investigation in Lower Austria. In: Geophysical Research Abstracts, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018-5066. https://meetingorganizer.copernicus.org/EGU2018/EGU2018-5066.pdf.

MABIT, L., FULAJTAR, E., TOLOZA, A., OCHOA, V., MAESTRONI, B.M. (2018 c). Implementation and optimization of soil sampling: some practical guidance and considerations. Chapter 4. In: *Integrated Analytical Approaches for Pesticide Management* - 1st Edition. Eds: B. Maestroni & A. Cannavan. pp. 47–63. Academic Press.

MABIT, L., GIBBS, M., MBAYE, M., MEUSBURGER, K., TOLOZA, A., RESCH, C., KLIK, A., SWALES, A., ALEWELL, C. (2018 d). Novel application of a Compound Specific Stable Isotope (CSSI) technique to investigate on-site sediment origins across arable fields. *Geoderma*, 316, 19–26.

MAO, Y., HEILING, M., ZOU, S., XING, S., ZHAO, Z., HUANG, Z. (2018). Effects of biochar from spent mushroom substrate on the physicochemical properties in a nutrient-poor soil. In: Geophysical Research Abstracts, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018-3961. https://meetingorganizer.copernicus.org/EGU2018/EGU2018-3961.pdf .

MARTIN, V., SUN, S., CANARINI, A., ZEZULA, D., JECMENICA, M., MACZALOVÁ, M., BOGDANOVIC, I., SIDRA, S., DERCON, G., BOECKX, P., WALKER, T., RICHTER, A. (2018). Microbial carbon dynamics along an elevational gradient in the central Austrian Alps. In: Geophysical Research Abstracts, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018-13276. http://meetingorganizer.copernicus.org/EGU2018/13276.pdf.

MARTINEZ, Y., MORERA, S., DERCON, G., NAVAS, A. (2018). Modeling Soil Erosion and sediment delivery to rivers in a mountainous tropical basin in the Peruvian Andes. In: Geophysical Research Abstracts, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018-1034. http://meetingorganizer.copernicus.org/EGU2018/1034.pdf.

MEUSBURGER, K., PORTO, P., MABIT, L., LA SPADA, C., ARATA, L., ALEWELL, C. (2018). Excess Lead-210 and Plutonium-239+240: Two suitable radiogenic soil erosion tracers for mountain grassland sites. *Environmental Research*, 160, 195–202.

MUSTAFA, O., DERCON, G., EVANGELISTA, H., KUDNIKOV, A., MAERCKER, J., MAVLYUDOV, B., NAVAS, A., SELIVERSTOV, Y., SLAETS, J., STOTT, T., YANGCHUN, W., PFEIFER, C. (2018). Monitoring proglacial geomorphological landforms with Unmanned Aerial Vehicles (UAVs). In: Geophysical Research Abstracts, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018-10025. http://meetingorganizer.copernicus.org/EGU2018/10025.pdf.

NAVAS, A., GASPAR, L., VAKHRAMEEVA, P., LIZAGA, I., MAVLYUDOV, B., STOTT, T., GERARDO-ABAYA, J., SLAETS, J., DERCON, G. (2018). Identifying the provenance of sediments in melting season in Artic Aldegonda Glacier (Svalbard). In: Geophysical Research Abstracts, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018-5996. http://meetingorganizer.copernicus.org/EGU2018/5996.pdf.

OAQUIM, A., EVANGELISTA, H., MOSER, G., DERCON, G., LICÍNIO, M., VIJVER, B. (2018). The use of lake diatoms from pro-glacial areas as climate proxy for the Northern Antarctic Peninsula since the 19th Century. In: Geophysical Research Abstracts, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018-3944. http://meetingorganizer.copernicus.org/EGU2018/3944.pdf.

RAZAQ, I. B., WAHBI, A. (2018). Introduction. In: *Challenges and Opportunities for Crop Production in Dry and Saline Environments in ARASIA Member States.* pp 1–3. IAEA-TECDOC-1841. IAEA publication.

SHAABAN, A.S.A., WAHBI, A., SINCLAIR T.R. (2018). Sowing date and mulch to improve water use and yield of wheat and barley in the Middle East environment. *Agricultural systems*, 165, 26-32.

TORRES ASTORGA, R., DE LOS SANTOS VILLALOBOS, S., VELASCO, H., DOMINGUEZ-QUINTERO, O., PEREIRA CARDOSO, R., MEIGIKOS DOS ANJOS, R., DIAWARA, Y., DERCON, G., MABIT, L. (2018). Exploring innovative techniques for identifying geochemical elements as fingerprints of sediment sources in an agricultural catchment of Argentina affected by soil erosion. *Environmental Science and Pollution Research*, 25(21), 20868–20879.

VELASCO, H., TORRES ASTORGA, R., JOSEPH, D., ANTOINE, J.S., MABIT, L., TOLOZA, A., DERCON, G., WALLING, D.E. (2018). Adapting the Caesium-137 technique to document soil redistribution rates associated with traditional cultivation practices in Haiti. *Journal of Environmental Radioactivity*, 183, 7–16.

VERKULICH, S., EVANGELISTA, H., PUSHINA, Z., DERCON, G. (2018). Holocene climate and environmental changes, recorded in terrestrial deposits and lake sediments on the Fildes Peninsula, King George Island (West Antarctica). In: Geophysical Research Abstracts, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018-7499, http://meetingorganizer.copernicus.org/EGU2018/7499.pdf.

VERKULICH, S., ZAZOVSKAYA, E., PUSHINA, Z., SAVELIEVA, L., SOLOVEVA, D., DEMIDOV, N., SHISHKOV, V., DERCON, G. (2018). The postglacial environmental changes in vicinity of the Barentsburg settlement (West Spitsbergen). In: Geophysical Research Abstracts, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018-7729, http://meetingorganizer.copernicus.org/EGU2018/7729.pdf.

VREUGDENHIL, M., WAHBI, A., PFEIL, I., STRAUSS, P., WAGNER, W. (2018). Validation of spaceborne soil moisture products using a cosmic ray neutron sensor in Petzenkirchen, Austria. In: Geophysical Research Abstracts, Volume 20, European Geosciences Union – General Assembly 2018. Abstract EGU2018-6201. https://meetingorganizer.copernicus.org/EGU2018/EGU2018-6201.pdf.

WAHBI, A, HENG, L, DERCON, G. (2018). Cosmic Ray Neutron Sensing: Estimation of Agricultural Crop Biomass Water Equivalent, Springer book (Open source). https://link.springer.com/content/pdf/10.1007%2F978-3-319-69539-6.pdf.

WAHBI, A., AVERY, W. (2018). In Situ Destructive Sampling. In: Cosmic Ray Neutron Sensing: Estimation of Agricultural Crop Biomass Water Equivalent, 5-9 (Eds Wahbi, A., Heng, L., and Dercon, G.) Springer (Open source). https://link.springer.com/chapter/10.1007/978-3-319-69539-6_2.

An Update on the ReNuAL Project:
the FAO/IAEA Agriculture & Biotechnology Laboratories

ReNuAL is the initiative to modernize the eight aging laboratories in Seibersdorf, Austria, that are managed by the IAEA's Department of Nuclear Science and Applications. These laboratories, five of which belong to the Joint FAO/IAEA Division, strengthen Member States' capacities to use nuclear and related techniques in food and agriculture, human health, the environment and scientific instrumentation.

The modernization began in 2014 with the Renovation of the Nuclear Applications Laboratories (ReNuAL) project, which consists of new building construction to provide new space to some of the laboratories, the acquisition of new laboratory equipment and infrastructure upgrades. ReNuAL is fully funded and completion of new facilities is approaching. The follow-up to ReNuAL, ReNuAL Plus (ReNuAL+), began in 2017 and will provide for additional construction, targeted refurbishment of the remaining laboratories, and further equipment.

Insect Pest Control Laboratory ready for transition in mid-August 2019

On 25 September 2017, IAEA Director General Yukiya Amano inaugurated the new Insect Pest Control Laboratory. Representatives of more than 35 Member States joined the celebration in Seibersdorf and toured the new laboratory. With over 1700 m2 of laboratory space, the new facility will substantially increase the ability of the Joint FAO/IAEA Division to assist Member States in controlling harmful insect pests. The new IPCL fit out is completed and building systems are operational. It will be accepted from the contractor and be ready for laboratory transition in mid-August 2019. It is expected that the relocation timeframe will be approximately 6 months. It is noteworthy to mention that the IPCL consists of 4 scientific groups, with 75 laboratory activities and more than 500 equipment items, and that staff, equipment and insects will all have to be moved simultaneously.

The new Insect Pest Control Building

Flexible Modular Laboratory on track

With the contract for full fit out awarded on 19th October 2018 and the fit out progressing as planned, the construction of the Flexible Modular Laboratory (FML) is on track to be completed in April 2020. It is anticipated that the relocation of the laboratories to their new facilities within the FML will start in May 2020.

Thanks to recent extra-budgetary contributions from Member States, funds raised for ReNuAL+ construction secured full funding for the Animal Production and Health Laboratory, the third planned laboratory of the FML. Construction of the first two laboratories is fully funded under ReNuAL.

The FML building in November 2018

Resource Mobilization

Overall, the target budget for ReNuAL/ReNuAL+ is estimated at €57 million. the IAEA has raised over €35 million in extra-budgetary funds plus in-kind contributions for ReNuAL and ReNuAL+ from 36 Member States and other donors; as well as €14 million through the regular budget, taking overall funds available for ReNuAL and ReNuAL+ to €49 million. Funding required to complete the initiative, from both regular budget and extrabudgetary funds, amounts to approximately €8 million.

In 2018, eleven Member Sates announced and overall pledge of €4.1 million, including €1 million outside ReNuAL+ scope. Out of this total pledge, €2.5 million are for the ReNuAL+, and a €1.25 million needs to be raised in order to achieve DG's September 2018 target of €3.75 million.

Contributors List as of February 2019

Donors were recognized at an event during the IAEA's General Conference that featured the unveiling of the ReNuAL/ReNuAL+ donor wall. Director General Amano delivered remarks along with representatives of the Co-chairs of the Friends of ReNuAL: Ms Thembisile C. Majola, Deputy Minister of Energy for South Africa, and Mr Thorsten Herdan, Director General for Energy Policy of the Federal Ministry for Economic Affairs and Energy of Germany. The donor wall will be permanently displayed in the new Insect Pest Control Laboratory in Seibersdorf.

Refurbishment of the remaining laboratories

The upcoming focus for resource mobilization is the enhancement and refurbishment - e.g. reconfiguring lab space, mechanical and technical upgrades, new stand-alone elements such as greenhouses - of the laboratories remaining in the current buildings, namely:

- Plant Breeding and Genetics Laboratory;
- Terrestrial Environment Laboratory;
- Nuclear Sciences and Instrumentation Laboratory; and
- Dosimetry Laboratory.

Equipment manufacturer donates to support food safety

Shimadzu Corporation, a commercial company, has come forward to support the enhancement of the laboratories in Seibersdorf: The Food and Environmental Protection Laboratory of the Joint FAO/IAEA Division will be able to increase its efforts to help Member States test for contaminants in food thanks to an in-kind donation of sophisticated detection equipment. Director General Amano signed the agreement for the donation with Shimadzu Chairman Mr Akira Nakamoto in Tokyo on 2 October 2017. The Food and Environmental Protection Laboratory will use the new machine to train scientists from all over the world in applying state-of-the-art analytical methods to test for contaminants, such as pesticides and veterinary drug residues, in basic food products. It will also support FAO/IAEA research on reliable methods to confirm the origin of food and to test for food adulteration. The donated machine is a liquid chromatograph with triple quadrupole mass spectrometric capabilities (LC-MS/MS).