Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

FAO/IAEA
Agriculture & Biotechnology
Laboratories

Activities Report **2021**
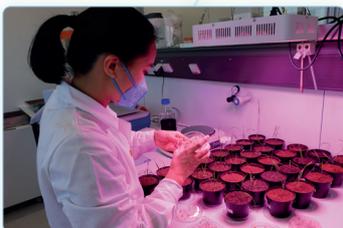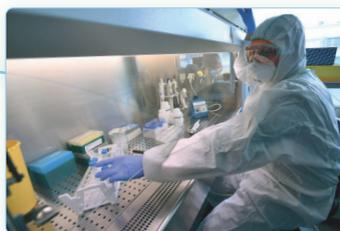
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FAO/IAEA

Agriculture and Biotechnology Laboratories

Activities Report 2021

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THE ANIMAL PRODUCTION AND HEALTH LABORATORY

EXECUTIVE SUMMARY

The main mandate of the Animal Production and Health Laboratory (APHL) of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture is to provide assistance to Member States (MSs) in improving productivity of livestock and preventing and controlling transboundary animal and zoonotic diseases (TAZDs). To fulfil its mandate, several activities and projects are conducted, including research and development projects, validation, verification and transfer of nuclear and nuclear-related methods for laboratory disease diagnostic and surveillance. In addition, to effectively assess, share and transfer the technologies and techniques, seminars, workshop and trainings are organized together with interlaboratory comparisons and field missions.

Despite the disruption and challenges posed by the COVID-19 pandemic, the above-mentioned activities have been maintained and implemented in 2021. Vaccines and vaccination represent one of the most reliable and effective tools to prevent and control animal infectious diseases and zoonoses, by limiting the spread of the infection, reducing the economic losses, preserving animal welfare and securing food. In many cases, in the medium to long term the use of vaccines also reduces the use of antimicrobials and the related burden of antimicrobial resistance.

The APHL is leading several research activities in this area, particularly in the development of alternative technologies such as beta- and gamma- irradiation for pathogens inactivation and production of safe, effective antigens for veterinary vaccines. Parallel to this, the APHL research program is exploring the use of irradiation as a nuclear application for the development of new vaccine formulations and products to strengthen immune responses, with focus on novel adjuvants and paraprobiotics. Furthermore, the development, validation and transfer of molecular- and immune- assays to assess the immune response to vaccines and infections is also an important part of the animal health APHL subprogram. Key progress in this area is presented in the present report.

The initiatives of the IAEA such as the Zoonotic Disease Integrated Action (ZODIAC) initiative, as well as projects, documents and initiatives launched by other international organizations highlighted the importance of the early detection, surveillance and monitoring of infectious diseases in (domestic and wild) animals to reduce the risk of spill over and major spread of pathogens in livestock and human population. In line with international veterinary and public health priorities, in 2021 the APHL continued to assist MS veterinary laboratories – particularly those operating with limited resources - in building and strengthening their capacity for early detection and control of transboundary animal diseases and zoonoses, increasing the laboratory preparedness for novel, emerging pathogens such as SARS-CoV2. Laboratory tools for the serological surveillance of zoonotic and animal pathogens have been developed and evaluated to make them available to MS. In this report, readers can find information on two multi-species serological assays based on a novel technique (the Luciferase Immuno-precipitation System, LIPS) developed for the sensitive detection of SARS-CoV2 antibodies in different animals. One ELISA assay was developed and evaluated for the application of a DIVA (Differentiating Infected and Vaccinated Animals) control strategy and serological surveillance of capripox virus infections in ruminants (Lumpy Skin Disease, Sheeppox, Goatpox).

A novel "family-based" approach has been developed for the detection and rapid identification of zoonotic viruses causing respiratory infections. Furthermore, data have been generated and shared regarding porcine circoviruses, an emerging group of viruses responsible for important economic losses for the swine intensive farming, a sector in constant growth worldwide, including developing countries.

Together with counterpart laboratories and VETLAB Network partners in Member States, the APHL has been very active in sharing and dissemination of scientific results through publications in peer-reviewed journals. The list of 2021 publications is included in this report.

Concerning animal genetics, the APHL made significant progress in research and development, particularly in testing and validating the multi-species camelid DNA chip for genomic evaluation of old and new world camels for increased productivity. The chip is ready for transfer to MS and for the use of camel breeders across Africa, Asia and Latin America. Significant progress was also made on whole genome radiation hybrid mapping with successful development of a framework map for dromedary camels. The APHL provided technical and scientific support for genomic evaluation of crossbred cattle in Bangladesh, detection of selection signatures related to high altitude adaptation in Peruvian cattle and genome wide association study for milk production in Serbian Holstein cattle. Twelve novel DNA markers associated with host resistance against gastro-intestinal parasites were identified in Argentinian sheep. These novel markers will be used in potential marker assisted national sheep breeding programs of member states. In 2021, significant achievements were also made towards the successful implementation of Global Action Plans on Animal Genetic Resources. As part of developing baseline reference data and global assessment of native zebu cattle of Asia and Africa, a comprehensive database was established for meta-analysis of more than 2700 cattle from 73 breeds/populations located across 17 countries.

In addition to R&D, the APHL was also involved in capacity building activities in IAEA and FAO Member States. The APHL actively supported the strengthening of molecular genetic laboratories in four countries (Cameroon, Eritrea, Mongolia and Indonesia). Two regional and one national training courses were successfully implemented with 61 participants from ten countries trained on bioinformatics analysis of large sets of livestock genome data.

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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Animal Health

Measuring a broader immune landscape in vaccinated chicken to identify correlates of protection

Measuring immune responses following vaccination is very important in assessing its efficacy. The classic of way measuring the efficacy of vaccines during its development stage is by conducting animal challenge studies. These challenge studies are costly and have an intense bio risk. The recent advances in immunology and related sciences have paved the way to understand the immune parameters that coincides with protection of animals upon vaccination which are termed "correlates of protection". Therefore, by measuring such correlates of protection the potency of a vaccine could be estimated without conducting challenge studies. Also, these parameters are used to identify the animals that are vaccinated and protected with vaccines that are in currently in the market. The gold standard of such immune correlates of protection has been antibodies raised against the pathogen induced by the vaccine. However, in the recent years there is accumulation of information that show other immune markers which are resulted from cell-mediated immunity may hold better correlates of protection. Only through examination of a wide array of markers could we identify such markers.

To assist vaccine research projects in chicken, APHL has undertaken the development of experimental protocols that will measure the expression of Avian immune markers during animal trials and when carrying out *in vitro* assays using chicken spleenocytes (APHL Newsletter 70, July 2019). The panel under development at Seibersdorf uses cytokine expression analysis by qPCR to add to the existing panels of ruminants and pigs that are available at APHL (Sassu *et al.,* 2020). Thirty-two targets including cytokines, pathogen pattern receptors, cell surface markers and three calibrator genes were selected from published reports and through communication with collaborators for optimization using spleenocytes that had been stimulated with phorbol 12-myristate 13-acetate (PMA) and Ionomycin in vitro for interleukin production. Control spleenocyte cultures were maintained in the same conditions as treated cells. RNA was subsequently extracted from cells and used in a qPCR assay using SYBR green that measures the targeted interleukins. The conditions for PCR cycling were adopted from previous work (Adams *et al.,* 2009). Activated spleenocytes displayed consistent signature melt curves for 22 of the chosen 32 targets including two calibrator genes (Fig. 1).

Figure 1. *Melt curve analysis of 20 Avian interleukin and surface marker targets along with beta-actin and GAPDH from a qPCR reaction carried out on chicken spleenocytes stimulated using PMA and Ionomycin. The single peaks confirmed that the designed primers amplified specific single products.*

This assay has subsequently been used for measuring immune marker expression in Chicken samples from vaccine trial against H9N2 low pathogenic Avian influenza in Chiken (Fig. 2). In this experiment, 2 candidate vaccines were tested: either irradiated inactivated or chemically (Formalin) inactivated was tested in two different routes (ocular nasal; ON or subcutaneous; SC). These experiments are ongoing, and data obtained thus far are shown here. Based on these data, we can find markers that aligns with the protection when challenged with the virus.


Figure 2. *Expression of various immune markers following vaccination against. Spleen samples were obtained two weeks following primary and booster vaccination either with irradiated inactivated through ocular nasal route (ON-Irradiated) or Formalin inactivated through ocular nasal route (ON-Formalin) or irradiated inactivated through sub cutaneous route (SC-Irradiated) or Formalin inactivated through sub cutaneous route (SC-Formalin) or non-vaccinated (control). Single cells were obtained from spleens ad were stimulated with the H9N2 avian flu virus. qPCR was then carried out on*

RNA which was extracted from stimulated spleen cells and transcribed into cDNA. Data is shown as fold changes against a house keeping gene.

References.

1. Elena L. Sassu, Richard T. Kangethe, Tirumala Bharani K. Settypalli, Tesfaye Rufael Chibssa, Giovanni Cattoli, Viskam Wijewardana. Development and evaluation of a real-time PCR panel for the detection of 20 immune markers in cattle and sheep. Veterinary Immunology and Immunopathology 2020; 227, 110092. https://doi.org/10.1016/j.vetimm.2020.110092.

2. Adams SC, Xing Z, Li J, Cardona CJ. Immune-related gene expression in response to H11N9 low pathogenic avian influenza virus infection in chicken and Pekin duck peripheral blood mononuclear cells. Mol Immunol. 2009 ;46(8-9):1744-9. https://doi.org/10.1016/j.molimm.2009.01.025.

Irradiated, replication-incompetent Lactobacilli ensure safety and preserve the metabolic activity while inducing diverse immune pathways

In recent years, safety concerns regarding the administration of probiotics led to an increased interest in developing inactivated probiotics, also called "paraprobiotics". Gamma-irradiation represents a promising tool that can be used to produce safe paraprobiotics by inhibiting replication while preserving the structure, metabolic activity and immunogenicity of bacteria. In this regard, APHL has conducted a study to evaluate the ability of four strains of lactic acid bacteria (LAB: Lacticaseibacillus casei, Lactobacillus acidophilus, Lactiplantibacillus plantarum and Lacticaseibacillus paracasei) to preserve the metabolic activity and the immune modulation of swine porcine peripheral blood mononuclear cells (PBMCs), following gamma irradiation or heat inactivation.

This study was aimed at investigating the immune modulatory functions of irradiated non-replicative, yet metabolically active LAB. It was found that irradiated LABs were able to preserve the immune modulatory blueprint of their live form and being different to heat-killed counterpart with some strains but not for all. Results show that at all three doses tested of gamma-irradiation (low, optimal minimum and high) the metabolic activity was preserved. Surprisingly, in most of the cases metabolic activity after irradiation was even higher than live bacteria in terms of redox potential and ATP production. Based on previous studies, we can hypothesize that the augmented metabolic activity of irradiated LAB can be seen as an effort performed by the cell to counteract the damages caused by ionizing radiation. In addition, it was found that the membrane structural integrity of irradiated LAB is similar to live cells inversely to the heat treated. In terms of immune modulatory capacity, two out of four strains (L. acidophilus and paracasei) were able to induce an overall gene expression similar to that of live LAB. On the other hand, it was also observed other outputs, such as higher degree of similarity between live and heat-killed (L. casei) LAB, or where both treatments led to a similar change in gene regulation differently from the viable state (L. plantarum). This variability is well-known in the literature and depends on key factors such as selection of LAB strain, dose, duration of the stimulation (incubation) and method of inactivation, among others. It was observed an interesting, varied mosaic of statistically significant differences in the regulation of some of the key genes involved in immune modulation that was analyzed. For instance, IFNα, mostly involved in anti-viral activity, was down-regulated by heat-killed L. casei, whereas IL-6, which is a pivotal pro-inflammatory cytokine responsible of regulating the immune response, playing a key role in stimulating B-cells differentiation, was up-regulated by live L. paracasei and gamma-irradiated L. casei. IL-21, another pro-inflammatory cytokine who play a role in Th17 development (which modulation seems to play an important role in

adjuvant development), proliferation of T-cells and differentiation of B-cells into memory cells, was found to be up-regulated in both gamma-irradiated L. acidophilus and in heat-treated L. paracasei. Live L. plantarum and heat-treated L. paracasei were able to up-regulate IL-23, which is one of the major effector molecules for Th17 maturation. Both gamma-irradiated treated L. acidophilus and L. paracasei induced down-regulation of the immunomodulatory TGFβ, which is mainly involved in suppressing T- and B-cells action while activating regulatory T (Treg) response. Finally, live L. acidophilus induced a down-regulation of Toll-like receptor 9 (TLR9), which have been proven to be essential for probiotics to exert an anti-inflammatory effect (Table 1). Ionizing radiation technology such as gamma or E- beam irradiation, is able to damage the nucleic acid of the organism, by inducing polymerization of the DNA and breaking molecular bonds, without affecting cell functions or the main components of the cells which are destroyed in other inactivation methods such as heat treatment. To confer an extra level of protection to the cell wall of the LAB used for this study, Trehalose was added to our samples prior to irradiation as a cryo- and radio-protectant. A positive correlation between the preservation of metabolic activity after gamma irradiation and immune stimulation capacity was highlighted in some studies, showing a better immunogenicity exerted by irradiated compared to heat-inactivated bacteria. In addition, studies have demonstrated that compounds of bacterial cells (e.g., teichoic acid, cell-wall polysaccharides, exopolysaccharides) are plausibly the main responsible for the pro- or anti-inflammatory effects exerted by these microorganisms and that the exposition of high temperatures due to the heat-treatment, induce a denaturation and coagulation of these proteins.

Strain / Treatment	IFNα	IL-6	IL-21	IL-23	TGFβ	TLR9
L. casei / Heat	↓ (*)	-	-	-	-	-
L. casei / Irradiation	-	↑ (**)	-	-	-	-
L. casei / Live	-	-	-	-	-	-
L. acidophilus / Heat	-	-	-	-	-	-
L. acidophilus / Irradiation	-	-	↑ (*)	-	↓ (*)	
L. acidophilus / Live	-	-	-	-	-	↓ (*)
L. plantarum / Heat	-	-	-	-	-	-
L. plantarum / Irradiation	-	-	-	-	-	-
L. plantarum / Live	-	-	-	↑ (*)	-	-
L. paracasei / Heat	-	-	↑ (*)	↑ (***)	-	-
L. paracasei / Irradiation	-	-	-	-	↓ (*)	-
L. paracasei / Live	-	↑ (*)	-	-	-	-

Table 1. Summary table of immune modulation exerted by immunobiotic and paraprobiotic LAB strains. *In this table, LAB strains, treated or untreated, are listed in the first column and the ones highlited in bold are able to induce a statistically significant difference in the up- (↑) or down-regulation (↓) of the immune markers listed in the first row. On the left side of the table is reported the similarities in overall gene expression modulation, observed in heat maps hierarchical clustering, where solid line shows similarity among different state of the same strain while dashed line shows similarity among live strains.*

This does not imply that an immune modulatory activity of the heat-treated strains is not expected, on the contrary studies have demonstrated that immune modulation can be even more pronounced, but that it would differ from the viable state. A way forward will be to select the most promising strains among the four investigated and conduct a kinetic analysis, including more time points of gene expression evaluation, to advance our understanding on how the gene expression of these immune markers is modulated at different time points, to elucidate mechanism of actions and especially on what are the short- and long-lasting effects, as can be a permanent up-regulation of a pro-inflammatory cytokine that if not regulated, could be detrimental for the organism. The ultimate goal is to identify a set of non-replicative metabolically active LAB strains that can confer a diverse immune stimulation, thus allowing the user to choose a specific strain based on the immune modulatory effect needed.

A novel Luciferase Immunoprecipitation System (LIPS) assay for SARS-CoV-2 antibody detection in different animal species

The recent emergence of SARS-CoV-2 in humans from a yet unidentified animal reservoir and the capacity of the virus to infect pets and farmed animals, and potentially wild animals, has highlighted the need for serological surveillance tools. APHL has evaluated the luciferase immunoprecipitation system (LIPS) for antibody detection in various animal species. This collaborative work involved the National Institutes of Health, Bethesda, Maryland, USA, the Instituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy, and the Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany).

Sera from SARS-CoV-2 naturally infected mink (n=77), experimentally SARS-CoV-2-infected ferrets, fruit bats, and hamsters, and a rabbit vaccinated with a purified spike protein were examined for antibodies using the SARS-CoV-2 nucleocapsid (N) and/or spike (S) proteins. From comparison with known neutralization status of the serum samples, statistical analyses, including calculation of the Spearman rank-order-correlation coefficient and Cohen's kappa agreement, were used to interpret the antibody results and diagnostic performance.

The LIPS immunoassay robustly detected viral antibodies in naturally infected SARS-CoV-2 mink and experimentally infected ferrets, fruit bats, and hamsters (Fig. 3). For the SARS-CoV-2-LIPS-S assay, there was a good level of discrimination between the positive and negative samples for each of the five species tested with 100% agreement with the virus neutralization results. In contrast, the SARS-CoV-2-LIPS-N assay did not consistently differentiate between SARS-CoV-2 positive and negative sera. This study, published in the journal Viruses (2021, 13, 1649, https://doi.org/10.3390/v13081649), shows the suitability of the SARS-CoV-2-LIPS-S assay for the sero-surveillance of SARS-CoV-2 infection in a range of animal species.
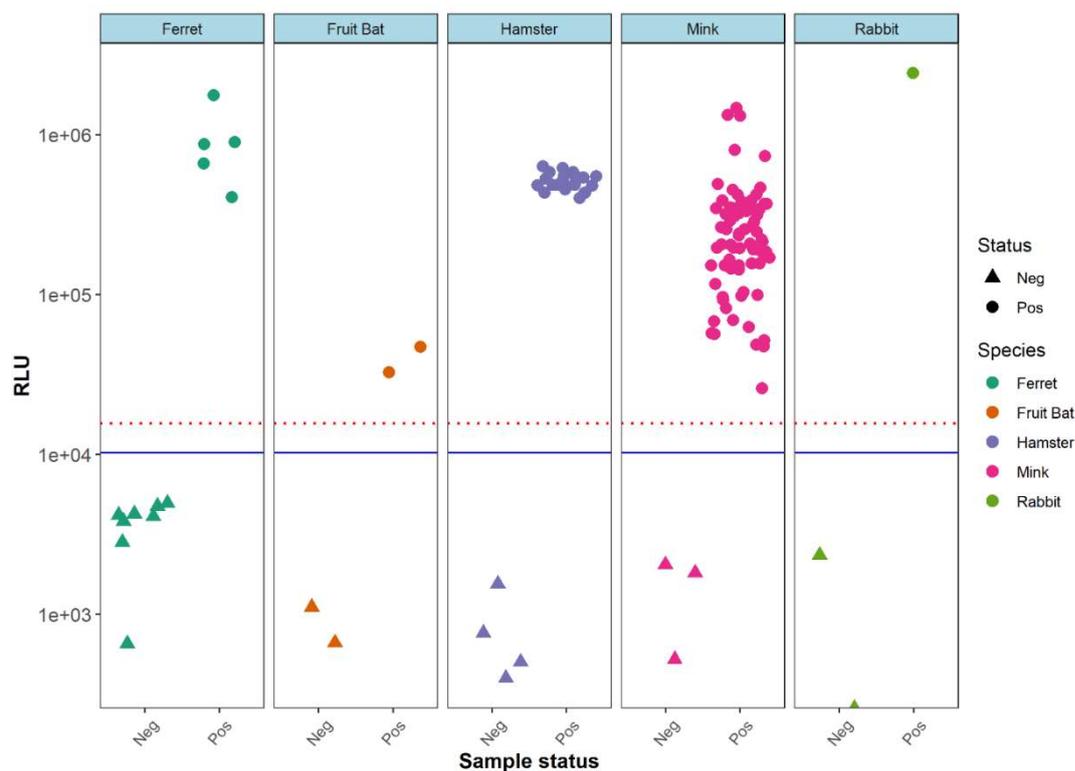
Figure 3. *Distribution of the LIPS-S assay antibody values based of the sample's known SARS-CoV-2 antibody neutralization status. Note, that all negative samples are located below the blue threshold line of mean plus 3 standard deviations and all positive samples are above the red dotted threshold line of mean plus 5 standard deviations.*

Family-based approach for direct detection of respiratory viral pathogens in clinical samples

Respiratory infections, especially those caused by viruses, are among the most common infectious diseases, globally. They are primarily confined in the upper respiratory tract, causing similar symptoms, including coughing, fever, runny nose, sneezing, and sore throat. However, some pathogens can also replicate in the lower respiratory tract and lead to complications such as pneumonia and bronchiolitis. As many of these pathogens originate from animal sources and can cross the species barrier, effective molecular surveillance in animals can help prevent the spillover from animals to humans. Hence, direct detection and identification of respiratory infecting viruses through syndromic surveillance or targeted surveillance in specific animal species required versatile tools. APHL has designed and evaluated an assay incorporating multiplex RT-PCR and nanopore sequencing technology to detect (Alpha- and Beta-) Coronaviruses and Orthomyxoviruses (influenza type A and D) families. Based on the optimized conditions, analyses of the PCR products on a 2 % agarose gel showed an upper band (~200 bp) representing Coronavirus and Influenza D virus, a lower band (~166 bp) for Influenza A virus, and no band on the negative control (NTC) (Figure XXX). To further identify and confirm the specificity of the assay, PCR products of a multiplexed sample (no. 9): (H3N2 (Infl-A) + HcoV-229E (Alpha) + SARS-CoV1 (Beta) + SARS-CoV2 (Beta) + Infl-D L6/17 + Infl-D L1/19) were purified and sequenced using Oxford nanopore technology (ONT). The obtained fastq sequences were analyzed using the EPI2ME software, which identified all the virus populations found in multiplexed samples (no. 9) (Fig. 4). Following the optimization, the assay will further be validated in the field.
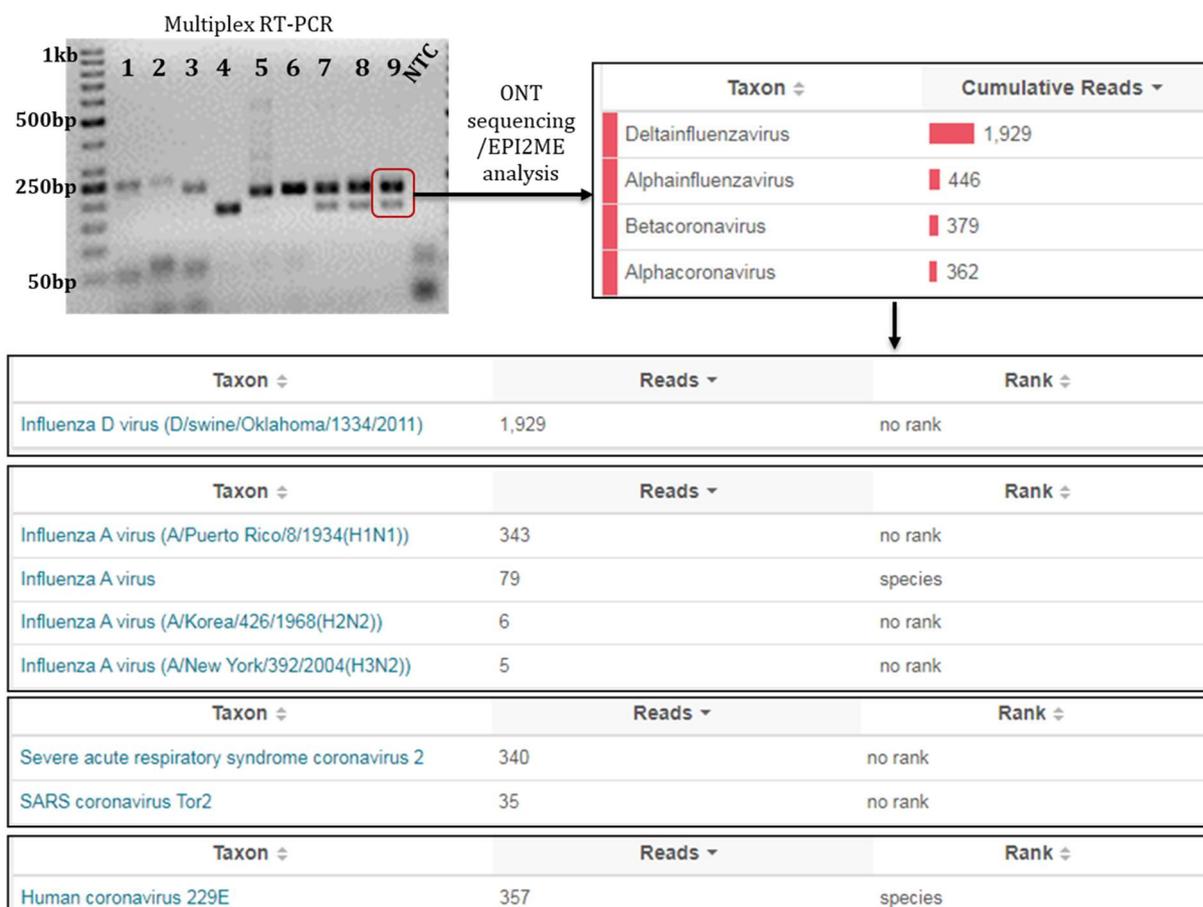
Figure 4. *Results of gel image showing the amplification pattern of different viruses in the multiplex RT-PCR assay, and the EPI2ME analysis output of the sequenced sample (in red block).*

A novel ELISA for Capripoxviruses for the serological differentiation of infected and vaccinated animals (DIVA test)

Capripoxviruses are causal agents of three economically relevant poxvirus diseases: sheeppox (SPP) and goatpox (GTP) affecting sheep and goats and lumpy skin disease (LSD) affecting cattle. SPP, GTP, and LSD are on the list of notifiable diseases by the World Animal Health Organization (OIE). These diseases affect small ruminants and cattle industries due to animal mortality, morbidity, low milk production, weight loss, abortions, and skin damage. SPP and GTP are endemic in most Africa, the Middle East, and Central Asia, including the Indian sub-continent. Most recently, LSDV has extended from Africa and the Middle East to parts of Europe, Russia, China and East Asia.

Vaccination is among the recommended methods to control these diseases, and live attenuated vaccines are available in the market. In countries using vaccination, it is essential to identify if an animal or herd has antibodies as part of the vaccination campaign for epidemiological and trade-related reasons or if it has been infected. At present, serological tests able to discriminate between vaccinated and infected animals are not available.

For this purpose, APHL has developed an ELISA based on a recently identified target in the capripoxvirus genome to specifically detect natural infection but not for the vaccine. The serological assay has shown 100% sensitivity and specificity when tested against 32 naturally infected, 78 vaccinated, and 73 negative cattle sera, as well as 27 naturally infected sheep and goat, 14 vaccinated

sheep, and 87 sheep and goat negative sera (Fig. 5). In addition, ORF, pseudocowpox, and bovine papular stomatitis samples (19) tested negative in the specificity study. The current ELISA will be helpful to determine animal vaccination status in trade-related practices and capripox surveillance and eradication campaigns.
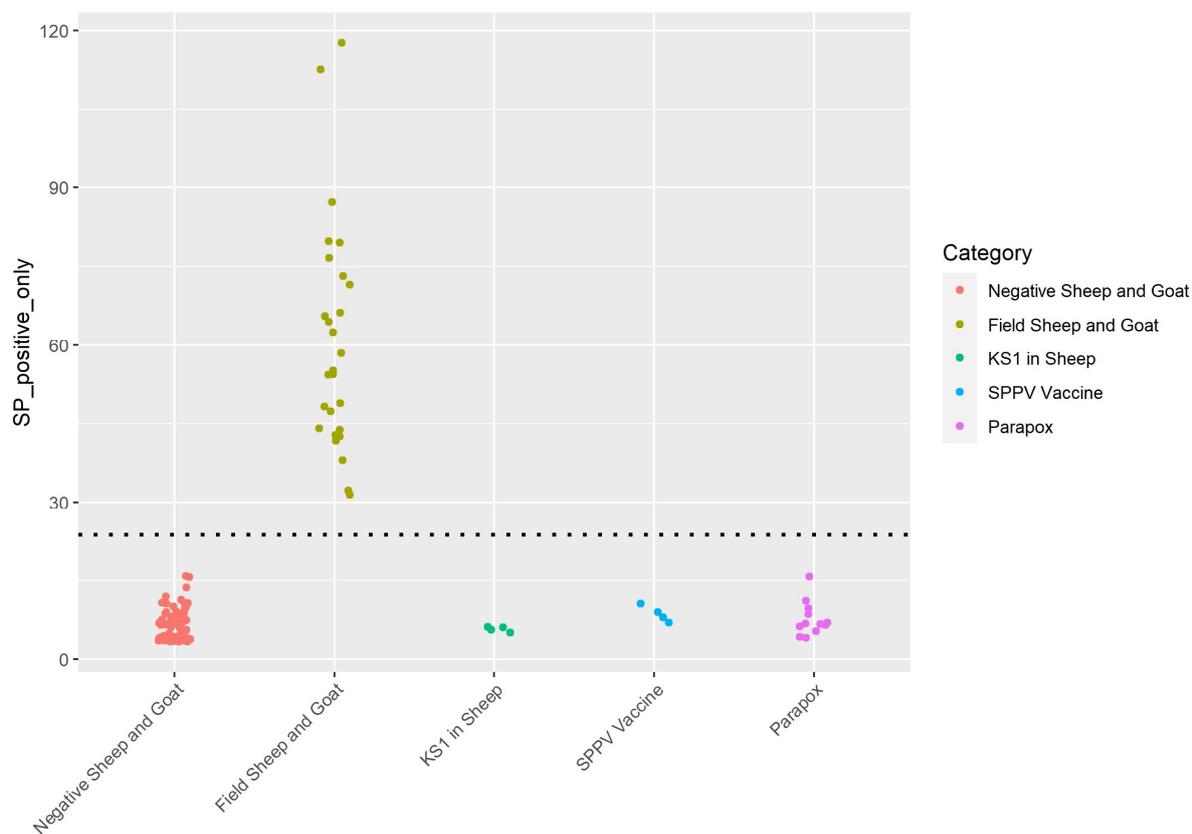

Figure 5. *Graph indicating naturally infected, vaccinated, negative and specificity control samples for sheep and goatpox. There is a clear separation between vaccinated and naturally infected samples.*

Studies on porcine circoviruses

The family Circoviridae, genus Circovirus includes four porcine circovirus species (i.e. PCV-1, PCV-2, PCV-3 and PCV-4) that are associated, to varying degrees, with disease in pigs. Porcine circovirus 2 (PCV-2) causes several syndromes globally defined as porcine circovirus diseases (PCVD) which includes postweaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS) and reproductive disorders. The virus is responsible for major losses for the swine industry, ascribable to both symptomatic and subclinical infections. PCV-3 was first identified in the USA and was linked to porcine dermatitis and nephropathy syndrome and reproductive failure, cardiac and multi-systemic inflammation in infected pigs. More recently, strong evidence of PCV-3's association with clinical disease in post-weaning pigs has also been provided. There is still some debate as to whether PCV-1 and PCV-4 cause disease. Due to the increasing intensification of the swine production on new areas of the world, there is a demand for more epidemiological data on the circulation of porcine circoviruses and their role in infections and immunity.

Little is known about porcine circoviruses in Africa and several studies were undertaken in APHL to determine the presence of PCV-2 in the African setting. Additionally, studies on identifying co-infections of PCVs and other porcine pathogens such as African Swine Fever were undertaken both on

samples from Africa and Asia. Data and scientific results were shared with member states and disseminated through scientific publications in peer-reviewed journals.

1. *PCV-2 in Africa: identification of continent-specific clusters and evidence of independent viral introductions from Europe, North America and Asia (doi: 10.1007/s00705-021-05035-9)*
2. *Co-infection of PCV-2 and African swine fever (doi: 10.1007/s00705-021-05312-7)*
3. *Co-infection of PCV-3 and African swine fever (doi: 10.1007/s11259-021-09858-4)*
4. *PCV-2 detected in the Oryx antelope (Oryx gazella) (doi: 10.3390/pathogens10111402)*

Animal Genetics

Testing and validation of multi-species microarray for genomic evaluation of camelids

In 2020, a multi-species camelid DNA chip was developed by Animal Production and Health Laboratory (APHL) in collaboration with Veterinary Medical University (Austria) and International Camel Genome Consortium. The chip consisted of ~200K markers with >60K from each of dromedary, Bactrian and new world camelid species. The array was tested and validated successfully to evaluate dromedary camels. During 2021, to validate the 60K Bactrian SNP (single nucleotide polymorphism) panel, 96 samples were tested to generate library files that can convert signal data into genotypes. The validation process was successful with extraction of genotypes at more than 51000 marker loci and a success rate of 86.2%. The thresholds for quality control parameters were set high with DQC>0.82, SNP QC call rate >97%, average call rate for passing samples ≥ 98.5 and percent passing samples ≥ 95. About 67.32% of genotyped markers were classified under PolyHigh Resolution (presence of both homozygotes and heterozygotes), 10.52% under NoMinor Homozygotes (absence of minor allele homozygotes) and 8.36% under MonoHigh Resolution (monomorphic) categories.

To validate the 60K new world camelid SNP (single nucleotide polymorphism) panel, 280 samples collected from four different new world camelid species (Alpaca, Llama, Vicugna and Guanaco) were analysed on the array. The raw signals were utilized to generate genotyping library files, specific for new world camelids. The validation process was successful with extraction of genotypes at more than 53000 marker loci and a success rate of 88.47%. The thresholds for quality control parameters were set high with DQC>0.82, SNP QC call rate >97%, average call rate for passing samples ≥ 98.5 and percent passing samples ≥ 95. About 30,400 (~50.7%) markers were classified under PolyHigh Resolution (presence of both homozygotes and heterozygotes) category, ~14100 markers (23.64%) under NoMinor Homozygotes (absence of minor allele homozygotes) category and ~8400 (~14%) under MonoHigh Resolution (monomorphic) category. The successful validation has now enabled genetic and genome wide evaluation of new world camelid species. The camelid array is now ready for transfer to member states and is also made available commercially from ThermoFisher-Affymetrix. Further genotyping of diverse old and new world camelid populations for biodiversity study is currently under progress.
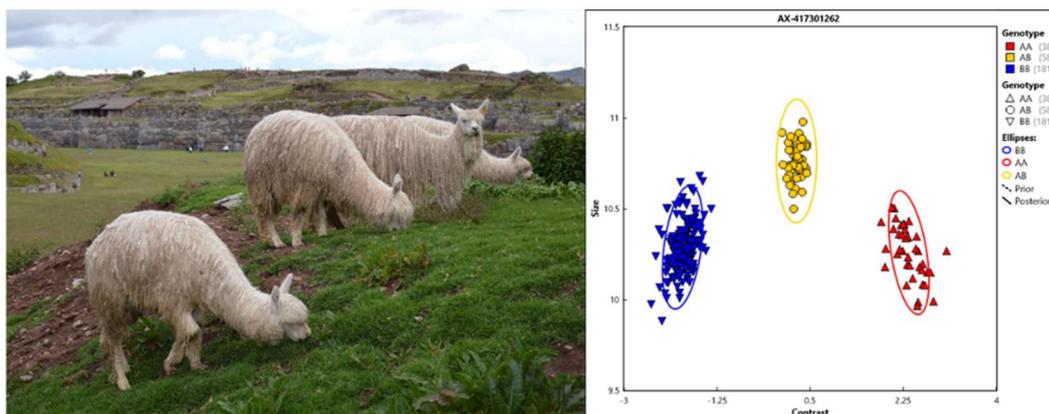
Marker Metrics Summary

- Number of Markers: 59995
- Number of BestandRecommended: 53077
- Percent BestandRecommended: 88.469

ConversionType	Count	Percentage
PolyHighResolution	30462	50.774
NoMinorHom	14181	23.637
MonoHighResolution	8434	14.058
Other	4410	7.351
OTV	1553	2.589
CallRateBelowThreshold	955	1.592

Figure 6. New world camelids of Peru (left); a sample new world camelid SNP cluster-plot from multi-species camelid microarray (right); Marker metric summary for new world camelid SNP panel

Whole genome radiation hybrid (RH) mapping of dromedary

Genomic resources such as whole genome linkage maps and reference genome assembly are scarcely available for camelid species. In 2018, the Joint FAO/IAEA laboratories at Seibersdorf completed the development of two radiation hybrid (RH) panels (5000_{RAD} and 15000_{RAD}) for dromedary camel to establish whole genome radiation hybrid maps. Experimental designs were formulated to characterize 5000_{RAD} RH panel using the multi species camelid array developed and validated at APHL. The summarized signal intensities were extracted from raw data using Axiom Analysis Suite. Various statistical approaches were considered for typing RH panels and K-Means clustering approach was selected to determine the presence or absence of a marker in the radiation hybrid clones. The RH genotypes for all the SNP (single nucleotide polymorphic) markers available on the array (Dromedary, Bactrian and Alpaca SNPs) were generated. The binary data (1-positive and 0-negative) for the 5000_{RAD} RH panel was generated and the input file for CarthaGene software was generated successfully. As a first step towards chromosome level mapping, SNP markers from dromedary alone were considered to construct the framework map. A stringent set of criteria on clustering parameters was applied to select vectors for the analysis. Resulting vectors were inspected visually and outliers removed manually. The markers were finally analyzed using CarthaGene software installed on a Linux platform to generate whole genome radiation hybrid maps. The markers that formed different linkage groups were compared with available genomic coordinates to establish chromosome level maps for dromedary.

Figure 7. *Framework map of dromedary chromosome 1 (LOD 10, total length of 1253.6 cR, on the left – RH distance in cR)*

Estimation of genetic admixture in Bangladeshi crossbred cattle

Improvement of cattle for milk production in Bangladesh occurs mainly through cross breeding programs. The CRP research contract on "Application of Nuclear and Genomic Tools for Genetic Improvement of Crossbred Friesian Cattle in Bangladesh" aimed (i) to determine the level of taurine admixture among crossbred cattle in different regions of Bangladesh using genome-wide SNP data and (ii) to associate production performance with different levels of taurine admixture under small holder production systems. APHL provided technical and scientific support to the project team for performing genome wide analysis and estimation of genetic admixture in crossbred cattle. A total of 1114 cattle (977 crossbreds, 79 purebred zebu and 58 purebred Holstein cattle) located in four administrative divisions of Bangladesh were genotyped using 60K bovine SNP array. The genotype data was used to estimate the level of taurine admixture and classify the crossbred cattle into six different groups: ≥87.5%, 75.0% to <87.5%, 62.5% to <75.0%, 50.0% to <62.5%, 25.0% to <50.0% &

<25.0% of taurine blood. The results revealed significant differences in the level of taurine admixture among crossbred cattle located in different regions of Bangladesh (Fig. 8). Phenotypic evaluation and comparison of performance (milk production and reproduction traits) among different crossbred genetic groups is currently under progress.
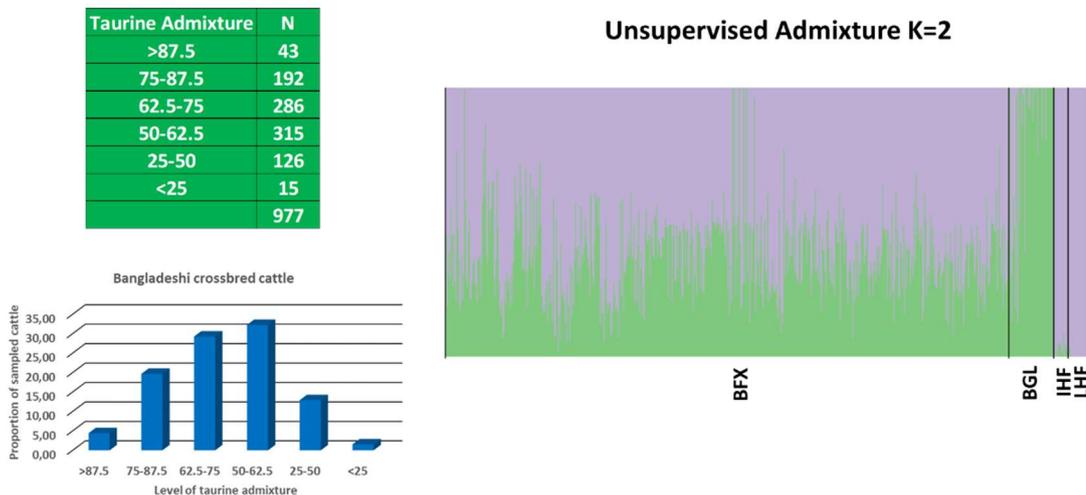
Figure 8. *Estimation of levels of taurine admixture in Bangladeshi crossbred cattle*

Detection of selection signature related to high altitude adaptation in Peruvian cattle

Cattle in Peru are managed in diverse production systems with varying climatic factors. Cattle are reared in high altitudes ranging from 1000 to 4800m above mean sea level (MSL). Over the period of years, the local Creole cattle has been graded up with Brown Swiss breed to improve milk and meat production, but still retaining the traits related to high altitude adaptation. The CRP research contract on "Genomic data from dairy cattle under different climatic conditions in Peru" aimed (i) to determine the level of genetic admixture in upgraded local cattle and (ii) to detect selection signatures related to high altitude adaptation in Peruvian cattle. APHL provided technical and scientific support to perform genomic evaluation and detection of selection signatures in Peruvian cattle. A total of 574 cattle (322 Brown Swiss, 191 Creole and 61 Holstein cattle) located in high and low altitude regions were genotyped using 60K bovine SNP array. The genotype data was used to estimate basic biodiversity measures, inbreeding, and genetic admixture levels in Peruvian cattle. The habitat of sampled cattle from each breed were classified as high and low altitude depending on their location. Two genome scan approaches based on extended haplotype homozygosity statistics (XP-EHH: Cross Population Extended Haplotype Homozygosity and Rsb scores) were utilized to detect signatures of selection (Fig. 9). A total of 28 gene ontology terms (e.g., chitin metabolic process, amino sugar catabolic process, etc.) were identified to be impacted among the high and low altitude Creole genomes. Further analysis of data is currently under progress.
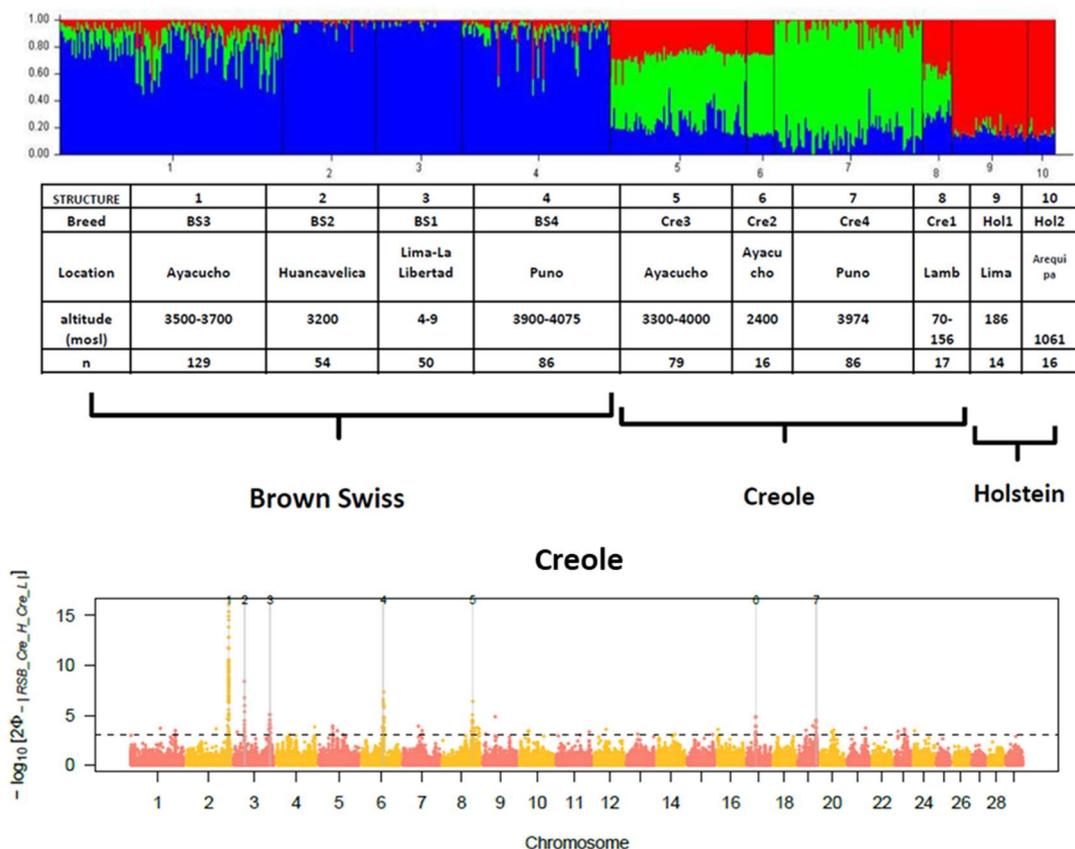
Figure 9. Genetic admixture and selection signature analysis in Peruvian cattle

Identification of novel gene markers associated with host resistance and resilience against gastro-intestinal parasites in Argentinian sheep

Selective breeding of genetically resistant animals is considered a promising strategy to face the problem of nematode resistance to anthelmintics and mitigate concerns about the presence of chemical residues in animal food products and the environment. Gastrointestinal nematode resistance is a complex, multifactorial trait related to host immunity. However, the mechanisms underlying host resistance and response to infection remain to be fully elucidated. In this context, a genetic association study was initiated by Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina in collaboration with APHL, Seibersdorf. The objective of this study was to provide insight into the chromosomal regions determining nematode resistance and resilience in Corriedale and Pampinta sheep breeds. A total of 170 single nucleotide polymorphic (SNP) markers from 76 candidate genes related to immune response were discovered by APHL using targeted resequencing approach. Real time PCR based genotyping assays for each of these markers were developed by APHL and transferred to INTA for genotyping and further analysis.

A total of 624 Corriedale and 304 Pampinta lambs underwent artificial or natural challenges with infective larvae mainly from *Haemonchus contortus*. Fecal egg counts, estimated breeding values for fecal egg counts, and rate of packed cell volume change and FAMACHA© score change over the challenge were used, when available, as indicators of host parasite resistance or resilience. Phenotype-genotype association studies were conducted. Eight SNPs, located on OARs 3, 6, 12, and 20, reached significance in Corriedale sheep under artificial challenge. Those SNPs represent allelic variants from the MHC-Ovine Lymphocyte Antigen-DRA, two C-type lectin domain families, the Interleukin 2 receptor β, the Toll-like receptor 10, the Mannan binding lectin serine peptidase 2, and the NLR family, CARD domain containing 4 genes. In Pampinta lambs under natural challenge, three significant SNPs located in the TIMP metallopeptidase inhibitor 3, the FBJ murine osteosarcoma viral

oncogene homolog, and the Interleukin 20 receptor alpha genes, on OARs 3, 7, and 8, respectively were identified. The novel DNA markers identified in the study can potentially be used for future marker assisted sheep breeding programs in Argentina.

Implementing Global Plan of Action for Animal Genetic Resources

In continuation of Joint FAO/IAEA efforts towards implementing Global Action Plan on animal genetic resources (AnGR), APHL supported member states in at least three major strategic priority areas: characterization, sustainable use and development and capacity building.

Meta-analysis of cattle from Asia, Africa and Europe

As part of developing baseline reference data and global assessment of native zebu cattle of Asia and Africa, several indigenous breeds were genotyped and sequenced under various coordinated research and technical cooperation projects. Data on short tandem repeat (STR) genotypes, genome-wide single nucleotide polymorphism (SNP) genotypes and mitochondrial control region sequences were generated to evaluate biodiversity, population structure, genetic admixture and genetic relationship of Asian, African and European cattle breeds. As a first step, STR data on >2700 cattle from 73 breeds/populations located across 17 countries were compiled on a master data file. Preliminary analysis on diversity and genetic relationship has been completed and further analysis on population structure and genetic admixture is in progress (Fig. 10).
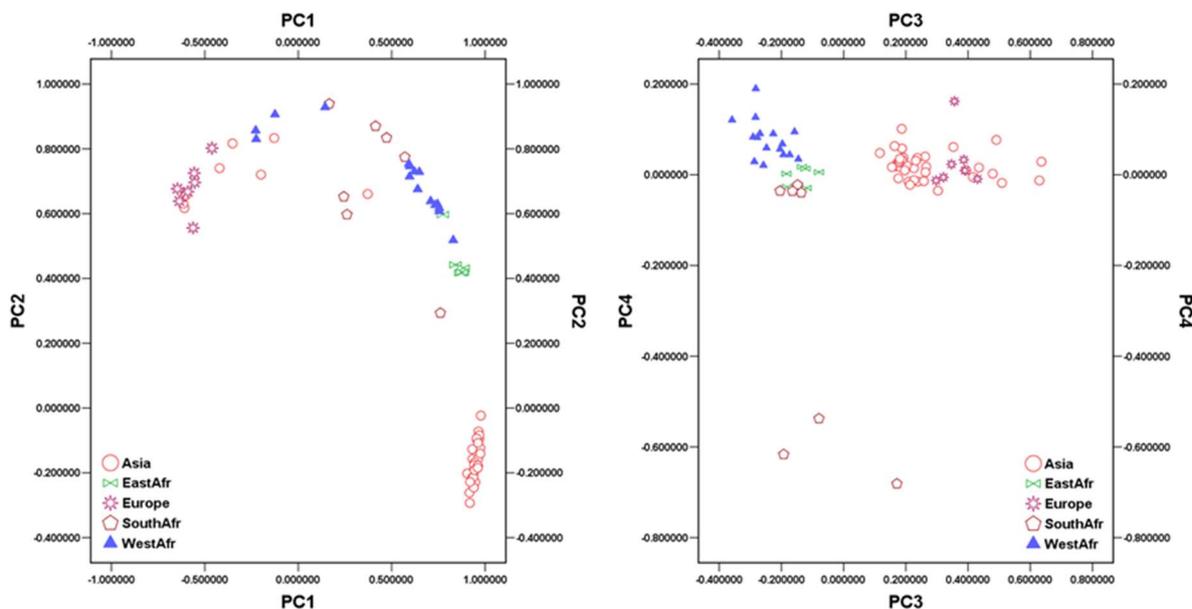
Figure 10. *Preliminary results on genetic relationship of cattle from Asia, Africa and Europe*

Population structure of endangered indigenous Jaffna sheep of Sri Lanka

Genetic diversity is an essential component in the fitness of a population, for it to survive and adapt to the changing environmental conditions. Baseline information on molecular genetic diversity provides insights in to breed history and helps in formulating strategies for conservation and genetic improvement of precious germplasm. Jaffna Local is an isolated and endangered indigenous sheep population in the island country of Sri Lanka. The native tract of Jaffna sheep has close geographic proximity with South Indian sheep breeds. Little or no information is available on diversity, population structure, historic admixture and South Indian ancestry in Jaffna sheep. Hence, University of Peradeniya, Sri Lanka initiated a study on Jaffna sheep in collaboration with Animal Production and Health Laboratory of the Joint FAO/IAEA Centre. The study was aimed (i) to evaluate genetic diversity of Jaffna Local sheep using short tandem repeat markers (ii) to estimate genetic relationships between Jaffna Local and South Indian sheep breeds (iii) to assess population structure and genetic admixture

in Jaffna Local sheep and (iv) to evaluate phylogenetic evolution and phylogeography of Jaffna local sheep based on mitochondrial DNA control region variations. A total of 235 sheep were genotyped at 19 short tandem repeat marker loci and sequenced for mitochondrial DNA (mtDNA) control region to assess population structure, genetic admixture and phylogeography.
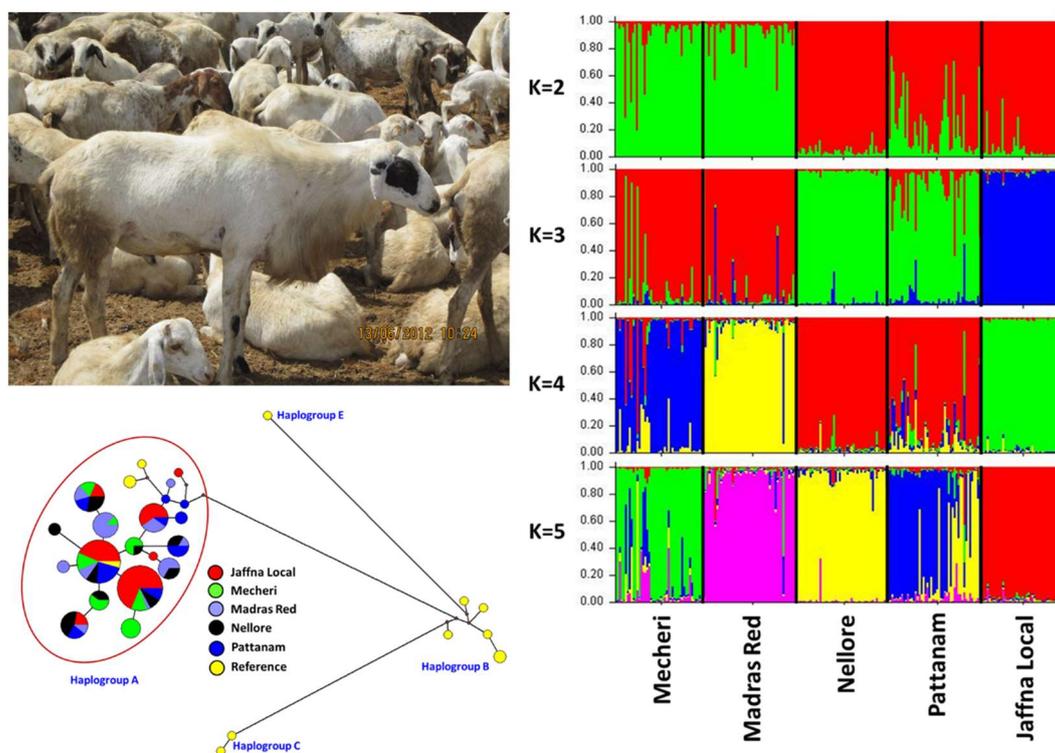
Figure 11. Population structure and phylogeography of endangered Jaffna sheep of Sri Lanka

The results revealed Jaffna Local sheep having relatively low diversity and high estimated inbreeding coefficient as compared to major South Indian breeds (Fig. 11). The pairwise F_{ST} showed Jaffna Local sheep having lowest genetic divergence with Pattanam sheep from South India and highest divergence with Mecheri and Madras Red sheep breeds. Bayesian clustering analysis with no prior population information also indicated very little gene flow from South Indian sheep into Jaffna Local population. Analysis of mtDNA control region revealed 16 haplotypes in Jaffna Local sheep, of which 10 were observed to be singletons and specific to this breed. The maternal lineages of all the Jaffna Local and South Indian sheep belonged to haplogroup A (HPG-A). The study clearly showed the genetic uniqueness of Jaffna Local sheep. It is imperative to implement selective breeding program in the native tract to prevent genetic dilution and foster conservation of this important indigenous genetic resource of Sri Lanka.

CAPACITY BUILDING

Strengthening laboratory infrastructure

APHL continued its efforts to improve the laboratory preparedness and capacity of member states. In 2021, APHL interventions enabled the implementation of advanced DNA based technologies for efficient management of locally available animal genetic resources. Equipment required to perform molecular genetic characterization were successfully installed, tested and validated at Seibersdorf laboratory. All the equipment were subsequently shipped to Animal Genetic Laboratories located in four countries viz. Cameroon, Eritrea, Mongolia and Indonesia. In the area of animal health, efforts were made to strengthen the capacity of veterinary laboratories in early detection and

characterization of transboundary animal and zoonotic diseases. RT-PCR equipment and reagents were provided to several countries in Africa, Asia and Caribbean regions facing the emergence of infectious diseases such as avian influenza, African Swine Fever, Lumpy Skin Disease and Peste des Petites Ruminants.

International and National Training Courses and Workshops

(i) Virtual Regional Training Course on "Genetic characterization of livestock breeds - Bioinformatics analysis of multi locus genotype data"

As part of Burkina Faso's National Action Plan on Animal Genetic Resources and the IAEA technical cooperation project (BKF5021) "Improving Local Poultry Production Through Incorporation of Nutraceuticals in Feeds and Genetic Characterization", a regional training course was organized from 06-10 July 2021 virtually. 26 participants from five countries in West Africa (7 from Burkina, 6 from Niger, 6 from Côte d'Ivoire, 4 from Senegal and 3 from Benin) attended the training course. The course included lectures and practical hands-on training on molecular genetic characterization and covered the following aspects: (i) Extraction of multi locus genotype data for genetic characterization of livestock breeds (ii) Estimate basic biodiversity indices of local breeds (iii) Estimate genetic distance and construct phylogeny (iv) Evaluate population structure and estimate levels of genetic admixture (v) Introduction to genome-wide technologies for genetic characterization of livestock breeds. The training is expected to help improve the regional capacity in applying molecular techniques for genetic characterization of indigenous livestock breeds in West Africa.

(ii) Virtual National Training Course on "Phenotype recording and conventional breeding methods for Cashmere goat improvement"

As part of Mongolia's national IAEA technical cooperation project (MON5025) "Improving Breed Characterization of Cashmere Goats to Facilitate the Establishment of Strategic Breeding Programs", a national training course was organized from 14-25 June 2021 virtually. Eleven participants from various institutions in Mongolia attended the training course. The course included lectures and practical hands-on training on: (i) Animal identification, phenotype recording and digitization of data in institutional farms and farmers' flocks (ii) Strategies for selection and breeding of goats to improve Cashmere wool production (iii) Introduction to basic concepts of quantitative genetics in animal breeding (iv) Estimation of genetic parameters and conventional breeding values (v) Introduction to genomics and potential applications for genetic improvement of livestock (vi) Applying genomics to estimate genetic distance, phylogeny, population structure and genetic admixture. The training course is expected to help improve the national capacity on performance data recording and breeding for cashmere wool production in Mongolia.

(iii) Virtual Regional Training on "Bioinformatics data analysis for biodiversity and genome-wide association studies in livestock"

As part of Mongolia's national IAEA technical cooperation project (MON5025) "Improving Breed Characterization of Cashmere Goats to Facilitate the Establishment of Strategic Breeding Programs", a regional training course was organized from 15-26 November 2021 virtually. 24 participants from five countries (Bangladesh, India, Mongolia, Pakistan and Sri Lanka) attended the training course. The course included lectures and practical hands-on training on: (i) Introduction to managing large sets of data using command line platforms: Basic Unix Commands (ii) Introduction to 'R' and working with 'R' Studio (iii) Introduction to PLINK and Data Quality Control (pruning genome wide single nucleotide polymorphic data (iv) Preparation of phenotype covariates and genotype data for genome wide association study (GWAS) (v) Estimation of inbreeding and estimating effective population size using genomic data (vi) Assessment of population structure and estimation of genetic admixture. The training is expected to help improve the regional capacity on handling large livestock genome datasets and utilizing genome wide information for biodiversity assessment, phenotype-genotype association studies, selection and breeding of local breeds for improved productivity.

iv) Consultancy Meeting on Development of Tools for the Mining, Monitoring and Tracing of Zoonotic Pathogens in Africa, the Americas & the Caribbean, Europe & Central Asia and Asia & the Pacific Areas – ZODIAC Collaborative Research Projects (CRPs)

Four virtual consultation meetings, one for each of the regions (Africa; Asia and Pacific; the Americas and Caribbean; Europe and Central Asia) were held between May and September 2021.
The meetings enabled discussion between external experts and key international organizations involved in zoonoses research and control, including the surveillance in diseases reservoirs and vectors and livestock in the respective regions. The purpose of these meetings was to identify priority zoonotic disease pathogens relevant for the region and explore and discuss on the tools needed for their mining, monitoring, tracing, and characterization to perform comprehensive field studies through multiple competent laboratories. Overall, the meetings enabled the participants to review and identify the best approaches and tools for sample collection, storage, disease surveillance, and pathogen mining that had the most significant potential for adaptation through the CRPs. As a result, the ZODIAC national laboratories will benefit from the tools and approaches of the CRPs to improve disease surveillance and monitoring in the countries and regions. The experts also identified critical R&D gaps that the CRPs could address and defined the priority diseases and pathogens and the essential species for inclusion in disease surveillance. The experts also drew up some recommendations to improve the research agenda of the CRPs.The meetings' outcomes formed the basis for the CRPs for each targeted region.

v) Virtual Preparatory Course on Proficiency Tests Organization and Management
In collaboration with the Enhancing Research for Africa Network (ERFAN), the VETLAB Network has supported the online preparatory course on organization and management of proficiency tests co-organized by the Botswana National Veterinary Laboratory (BNVL) in Gaborone and the Central Veterinary Laboratory in Harare-Zimbabwe. Ninety-four participants from 24 countries attended this on-line training consisting of once-a-week training sessions from 27th April to 8th June 2021.
The scope of the course was to introduce the basic concepts and terminology as well as ISO standards and international guidelines to organize and conduct interlaboratory comparison and prepare participants to the on-site course expected to be held as soon as COVID-19 restrictions allow. The final aim is to enable participants to acquire laboratory competences linked to the organization of Ring test for serological, bacteriological and molecular assays.

vi) Virtual Training Course for Veterinary Diagnostic Laboratory Network Partners on Sequencing and Bioinformatics
 The aim of the training course was to provide basic knowledge on next-generation sequencing (NGS) and NGS data analysis on the Linux interface. In addition, the participants received training on advanced concepts of the phylogenetic analysis of viruses. The training course for Veterinary Diagnostic Laboratory Network Partners on Sequencing and Bioinformatics took place virtually from 29 November to 10 December 2021. The two-week training course was attended by eighteen participants from 9 countries. Prior to the training course, the participants were assisted on a bilateral level with the set-up of a virtual Linux machine, which enabled them to run hands-on exercises on provided datasets.

vii) Peste des Petits Ruminants Global Eradication Programme Expert Group Workshop

In the context of the Peste des Petits Ruminants (PPR) Global Eradication Program (GEP), the Animal Production and Health Laboratory (APHL) of the Joint FAO/IAEA Centre, in collaboration with the Royal Veterinary College (UK) and the participation of the OIE/FAO PPR GEP Secretariat, organized a virtual PPR Expert Group Workshop. The aims of the workshop were as follows: i) to review the data and current state of knowledge on PPR that has been or could be derived from serological surveillance, and ii) to advise participants on what constitutes effective serological assays across host species, as well as on appropriate testing protocols or algorithms for populations during disease surveillance or monitoring for various purposes (e.g. general surveillance, post vaccination monitoring of viral circulation, specific host surveillance etc.). The workshop offered an opportunity to discuss the state of the art of PPR serology in wildlife and atypical hosts (e.g. camels and pigs). Moreover, the participants were introduced to the application of novel bELISA and serological platforms for PPR, such as LIPS and pseudo-type virus neutralization assay (PVNA) followed by a presentation and comparison of the results obtained from these assays. Twenty-five experts from international organizations (AU-PANVAC, FAO, IAEA, and OIE), veterinary research institutions and PPR reference laboratories participated in the workshop.

PUBLICATIONS

1. M.A. Raschia, M.V. Donzelli, P.D. Medus, B.M. Cetrá, D.O. Maizon, V.H. Suarez, R. Pichler, Kathiravan Periasamy and M.A. Poli (2021). Single nucleotide polymorphisms from candidate genes associated with nematode resistance and resilience in Corriedale and Pampinta sheep in Argentina. Gene, 770: 145345.

2. Kurukulasuriya, M. S., Notter, D. R., Silva, P., Periasamy, K., Pichler, R., Dematawewa, C. M. B., … Lokugalappatti, L. G. S. (2021). Crossing indigenous goats with a larger imported breed increases vulnerability to natural infection with gastrointestinal strongyle parasites. Livestock Science, 104359. doi:10.1016/j.livsci.2020.104359.

3. Manomohan V, Saravanan R, Pichler R, Murali N, Sivakumar K, Sudhakar K, Nachiappan RK, Periasamy K. Legacy of draught cattle breeds of South India: Insights into population structure, genetic admixture and maternal origin. PLoS One. 2021 May 24;16(5):e0246497. doi: 10.1371/journal.pone.0246497.

4. Kurukulasuriya M, Silva P, Pichler R, Thiruvenkadan AK, Saravanan R, Periasamy K. (2022). Population structure and genetic relatedness of Sri Lankan Jaffna Local sheep with South Indian breeds. *Small Ruminant Research,* 206, 106571.

5. **Viljoen, G.J.**, Unger, H., **Wijewardana, V.**, **Naletoski, I.**, "Novel developments and next-generation vaccines", Veterinary vaccines. Principles and applications (Metwally, S., Idrissi, A.E., Viljoen, G., Eds), John Wiley & Sons (2021) 119-134. DOI:10.1002/9781119506287.ch10.

6. **World Organisation for Animal Health (OIE)**, "Chapter 2.1.2. Biotechnology advances in the diagnosis of infectious diseases", Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2021. OIE (2021).

7. Anahory, I.V., Franzo, G., **Settypalli, T.B.K.**, Mapaco, L.P., Achá, S.J., Molini, U., **Cattoli, G., Lamien, C.E.**, Dundon, W.G., Identification of porcine circovirus-3 in Mozambique. Vet Res Commun. Nov 8 (2021). DOI: 10.1007/s11259-021-09858-4.

8. Ankhanbaatar, U., Sainnokhoi, T., **Settypalli, T.B.K.**, Datta, S., Gombo-Ochir, D., Khanui, B., Dorj, G., Basan, G., **Cattoli, G.**, Dundon, W.G., **Lamien, C.**, Isolation and Identification of a Highly Pathogenic Avian Influenza H5N6 Virus from Migratory Waterfowl in Western Mongolia. J Wildl Dis. Oct 26 (2021). DOI: 10.7589/JWD-D-21-00032.

9. **Berguido, F.J.**, Burbelo, P.D., Bortolami, A., Bonfante, F., Wernike, K., Hoffmann, D., Balkema-Buschmann, A., Beer, M., **Dundon, W.G., Lamien, C.E., Cattoli, G.**, Serological detection of SARS-

CoV-2 antibodies in naturally-infected mink and other experimentally-infected animals. Viruses **13** 1649 (2021). DOI: 10.3390/v13081649.

10. Chibssa, T.R., **Kangethe, R.T.**, **Berguido, F.J.**, **Settypalli, T.B.K.**, Liu, Y., Grabherr, R., Loitsch, A., Sassu, E.L., **Pichler, R.**, **Cattoli, G.**, Diallo, A., **Wijewardana, V.**, **Lamien, C.E.,** Innate Immune responses to wildtype and attenuated sheeppox virus mediated through RIG-1 sensing in PBMC in-vitro. Frontiers in Immunology **12** (2021)**.** DOI: 10.3389/fimmu.2021.666543.

11. Dundon, W.G., Franzo, G., Settypalli, T.B.K., Dharmayanti, N.L.P.I., Ankhanbaatar, U., Sendow, I., Ratnawati, A., Sainnokhoi, T., Molini, U., Cattoli, G., Lamien, C.E., Evidence of coinfection of pigs with African swine fever virus and porcine circovirus 2. Archives of Virology (2021). DOI: 10.1007/s00705-021-05312-7.

12. Franzo, G., **Settypalli, T.B.K**., Agusi, E.R., Meseko, C., Minoungou, G., Ouoba, B.L., Habibata, Z.L., Wade, A., de Barros, J.L., Tshilenge, C.G., Gelaye, E., Yami, M., Gizaw, D., Chibssa, T.R., Anahory, I.V., Mapaco, L.P., Achá, S.J., Ijomanta J., Jambol, A.R., Adedeji, A.J., Luka, P.D., Shamaki, D., Diop, M., Bakhoum, M.T., Lo, M.M., Chang'a, J.S., Magidanga, B., Mayenga, C., Ziba, M.W., Dautu, G., Masembe, C., Achenbach, J., Molini, U., **Cattoli, G., Lamien, C.E.**, Dundon, W.G., Porcine circovirus-2 in Africa: Identification of continent-specific clusters and evidence of independent viral introductions from Europe, North America and Asia. Transbound Emerg Dis. **Nov 23** (2021). DOI: 10.1111/tbed.14400.

13. Minoungou, G.L., Diop, M., Dakouo, M., Ouattara, A.K, **Settypalli, T.B.K.**, Lo, M.M., Sidibe, S., Kanyala, E., Kone, Y.S., Diallo, M.S., Ouedraogo, A., Coulibaly, K., Ouedraogo, V., Sow, I., Niang, M., Achenbach, J.E., Wade, A., Unger, H., Diallo, A., **Cattoli, G., Lamien, C.E.**, Simpore, J., Molecular characterization of African Swine fever viruses in Burkina Faso, Mali, and Senegal 1989–2016. Genetic diversity of ASFV in West Africa. Transbound Emerg Dis **68** 2842–2852 (2021). DOI: 10.1111/tbed.14240.

14. Modise, B.M., **Settypalli**, **T.B.K.**, Kgotlele, T., Xue, D., Ntesang, K., Kumile, K., **Naletoski, I.**, Nyange, J.F., Thanda, C., Macheng, K.N., Marobela-Raborokgwe, C., **Viljoen, G.J.**, **Cattoli, G., Lamien, C.E.**, First molecular characterization of poxviruses in cattle, sheep, and goats in Botswana. Virology Journal **18** 167 (2021). DOI:10.1186/s12985-021-01634-9.

15. Nooruzzaman, M., Akter, M.N., Begum, J.A., Begum, S., Parvin, R., Giasuddin, M., Islam, M.R., **Lamien, C.E., Cattoli, G.**, Dundon, W.G., Chowdhur,y E.H., Molecular insights into peste des petits ruminants virus identified in Bangladesh between 2008 and 2020. Infect Genet Evol. Nov 27 (2021). DOI: 10.1016/j.meegid.2021.105163.

16. Tuppurainen, E., Dietze, K., Wolff, J., Bergmann, H., Beltran-Alcrudo, D., Fahrion, A., **Lamien, C.E.**, Busch, F., Sauter-Louis, C., Conraths, F.J., De Clercq, K., Hoffmann, B., Knauf, S., Review: Vaccines and vaccination against lumpy skin disease. Vaccines **9** 10 (2021). DOI:10.3390/vaccines9101136.

17. Pawęska, J.T., Vuren, P.J. van, Msimang, V., Lô, M.M., Thiongane, Y., Mulumba-Mfumu, L.K., Mansoor, A., Fafetine, J.M., Magona, J.W., Boussini, H., Bażanow, B., Wilson, W.C., Pepin, M., Unger, H., **Viljoen, G.J.,** Large-Scale International Validation of an indirect ELISA based on recombinant nucleocapsid protein of Rift Valley fever virus for the detection of IgG antibody in domestic ruminants. Viruses **13** 1651 (2021). DOI: 10.3390/v13081651.

18. Ward, M.P., Tian, K., **Nowotny, N.,** African Swine fever, the forgotten pandemic. Transbound Emerg Dis **68** 2637-2639 (2021). DOI: 10.1111/tbed.14245.

19. Dundon WG, Settypalli TBK, Spiegel K, Steinrigl A, Revilla-Fernández S, Schmoll F, Naletoski I, Lamien CE, Cattoli G. Comparison of eleven in vitro diagnostic assays for the detection of SARS-

CoV-2 RNA. J Virol Methods. 2021 Jun 1; 295:114200. doi: 10.1016/j.jviromet.2021.114200. Epub ahead of print.

20. Chibssa TR, Sombo M, Lichoti JK, Adam TIB, Liu Y, Elraouf YA, Grabherr R, Settypalli TBK, Berguido FJ, Loitsch A, Sahle M, Cattoli G, Diallo A, Lamien CE. Molecular Analysis of East African Lumpy Skin Disease Viruses Reveals a Mixed Isolate with Features of Both Vaccine and Field Isolates. Microorganisms. 2021 May 26; 9(6):1142. doi: 10.3390/microorganisms9061142.

21. Molini U, Marruchella G, Matheus F, Hemberger YM, Chiwome B, Khaiseb S, Cattoli G, Franzo G. Molecular Investigation of Porcine Circovirus Type 3 Infection in Pigs in Namibia. Pathogens. 2021 May 11;10(5):585. doi:10.3390/pathogens10050585.

22. Chibssa TR, Liu Y, Sombo M, Lichoti JK, Erdenebaatar J, Boldbaatar B, Grabherr R, Settypalli TBK, Berguido FJ, Loitsch A, Damena D, Cattoli G, Diallo A, Lamien CE. Use of an Alignment-Free Method for the Geographical Discrimination of GTPVs Based on the GPCR Sequences. Microorganisms. 2021 Apr 16;9(4):855. doi: 10.3390/microorganisms9040855.

23. Molini U, Boshoff E, Niel AP, Phillips J, Khaiseb S, Settypalli TBK, Dundon WG, Cattoli G, Lamien CE. Detection of Lumpy Skin Disease Virus in an Asymptomatic Eland (Taurotragus oryx) in Namibia. J Wildl Dis. 2021 May doi: 10.7589/JWD-D-20-00181.

24. Ankhanbaatar U, Sainnokhoi T, Khanui B, Ulziibat G, Jargalsaikhan T, Purevtseren D, Settypalli TBK, Flannery J, Dundon WG, Basan G, Batten C, Cattoli G, Lamien CE. African Swine Fever Virus Genotype II in Mongolia, 2019. Transbound Emerg Dis. 2021 Apr 5. doi: 10.1111/tbed.14095.

25. Dharmayanti NI, Sendow I, Ratnawati A, Settypalli TBK, Saepulloh M, Dundon WG, Nuradji H, Naletoski I, Cattoli G, Lamien CE. African swine fever in North Sumatra and West Java provinces in 2019 and 2020, Indonesia. Transbound Emerg Dis. 2021 Mar 16. doi:10.1111/tbed.14070.

26. Molini U, Franzo G, Gous L, Moller S, Hemberger YM, Chiwome B, Marruchella G, Khaiseb S, Cattoli G, Dundon WG. Three different genotypes of porcine circovirus 2 (PCV-2) identified in pigs and warthogs in Namibia. Arch Virol. 2021 Mar 15. doi: 10.1007/s00705-021-05035-9.

27. Badhy SC, Chowdhury MGA, Settypalli TBK, Cattoli G, Lamien CE, Fakir MAU, Akter S, Osmani MG, Talukdar F, Begum N, Khan IA, Rashid MB, Sadekuzzaman M. Molecular characterization of lumpy skin disease virus (LSDV) emerged in Bangladesh reveals unique genetic features compared to contemporary field strains. BMC Vet Res. 2021 Jan 29;17(1):61. doi: 10.1186/s12917-021-02751-x.

28. Dellicour S, Lemey P, Artois J, Lam TT, Fusaro A, Monne I, Cattoli G, Kuznetsov D, et al. 2020. Incorporating heterogeneous sampling probabilities in continuous phylogeographic inference - Application to H5N1 spread in the Mekong region. Bioinformatics 36: 2098-2104. doi: 10.1093/bioinformatics/btz882

29. Fine AE, Pruvot M, Benfield CTO, Caron A, Cattoli G, Chardonnet P, Dioli M, et al. 2020. Eradication of peste des petits ruminants virus and the wildlife livestock interface. Front Vet Sci. 7: 50. doi: 10.3389/fvets.2020.00050

30. Souley MM, Issa Ibrahim A, Sidikou D, Dundon WG, Cattoli G, Abdou A, Soumana F, Yaou. 2020. Molecular epidemiology of peste des petits ruminants in Niger: An update. Transbound Emerg Dis 67: 1388-1392. doi: 10.1111/tbed.13451

Fellowship and internship training

In 2021, due to safety and health regulations related to the COVID-19 pandemic, APHL hosting capacity was limited to 1 fellow and 2 interns.

Name	Country	Status	Duration	Topic
Boitumelo Modise	Botswana	Fellow	12 months	Syndromic surveillance and molecular detection of zoonotic pathogens
Fatima Liaqat	Pakistan	Intern	12 months	Veterinary immunology and irradiated vaccines
Hanifati Subki	Indonesia	Intern	12 months	Implementation of the iVetNet platform

VETLAB NETWORK

The Veterinary Diagnostic Laboratory (VETLAB) Network, coordinated by the Animal Production and Health Section (APH) and supported through IAEA and FAO programmatic activities as well as by South Africa through the African Renaissance Fund (ARF) and by the USA and Japan Peaceful Uses Initiative (PUI), consists of national veterinary diagnostic laboratories located in 46 African and 19 Asia and Pacific Member States. Despite all the restrictions and limitations imposed by the pandemic situation, in 2021 the VETLAB Network has been very active and supportive with several activities being conducted. Through the very active contributions of network's partners, several scientific papers have been published, sharing relevant data and information on important transboundary animal diseases such as African Swine Fever (ASF), Pest des Petites Ruminants (PPR) and Lumpy Skin Disease (LSD), as well as on zoonotic diseases such as COVID-19 and avian influenza. Furthermore, the VETLAB Network provided emergency support and technical assistance to the African and Asian countries affected by H5N1 avian influenza virus, ASF and LSD.

It was with great pleasure that the network was able to organize this year the annual meeting of the directors of the VETLAB Network laboratories and an advanced VETLAB Training in Bioinformatics. Although the pandemic situation imposed the organization of these events on-line, it represents a positive step forward and demonstrates the continuous efforts of the network to strengthen laboratory capacity and preparedness.

Furthermore, the Network has organized the yearly interlaboratory trial for the serological and molecular detection of Peste des Petites Ruminants (PPR) virus. Twenty-nine laboratories from 27 countries accepted the invitation to participate and shipment of the panels is in due course. Due to the current epidemiological situation in the East Asian region, this year a panel for LSDV molecular detection is added for some Asian laboratories.

APH is issuing on a regular basis the VETLAB Network Bulletin in the hope of providing 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.

THE FOOD SAFETY AND CONTROL LABORATORY

EXECUTIVE SUMMARY

The Food and Environmental Protection Laboratory (FEPL) of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture assists Member States to implement and improve existing food control systems. This activity helps to ensure the safety and quality of the food supply, protecting consumer health and facilitating international trade. Technical support is provided for food authenticity and provenance determination and for the control of chemical residues and contaminants in food. This assistance and support underpins food safety and control systems and helps to safeguard human health and to identify and deter economic loss due to food fraud - the illegal and intentional marketing of counterfeit food products, intended to deceive consumers, for economic gain. Activities include applied and adaptive research and the development, validation, and transfer of nuclear and complementary analytical methods for testing foods. The application of these technologies and methods in Member States is supported through the development and provision of technical protocols, advice and guidance, training, and contributions to the development of international standards.

Research and development outputs were maintained in 2021 despite disruption caused by the COVID-19 pandemic. Method development encompassed both rapid screening methods and more sophisticated techniques, in order to provide Member States with the options needed for their food control systems. A first full year of activity in the new laboratory facilities of the Yukiya Amano Building has permitted us to consolidate a number of activities. Although laboratory outputs were, inevitably, impacted during the year by the continued uncertainties associated with COVID-19 pandemic travel restrictions, the capability of FEPL to assist Member States in the future has been greatly enhanced by the improved facilities.

Research and development work on analytical methods for the authentication of foods labelled as 'organic' continued in 2021. Over the past decade there has been a rapid increase in the total area of organic food production worldwide. However, this increase has been accompanied by increasing organic food fraud through misrepresentation. Methods were developed using compound-specific stable isotope analysis, in collaboration with the Isotope Hydrology Laboratory, to distinguish between organic and conventionally cultivated strawberries and bench-top nuclear magnetic resonance (NMR) spectroscopy was used with chemometrics, to discriminate between organic and conventionally produced orange juices. The analytical capabilities of FEPL were enhanced by the installation of a new bench-top Energy Dispersive X-Ray Fluorescence (EDXRF) spectrometer, which has many potential applications for food safety and authenticity screening. A new method was developed for the differentiation of Arabica and Robusta coffee beans by Multispectral Imaging (MSI). Furthermore, a new method was developed for the determination of the geographical origin of premium Hom Mali rice cultivated in different regions of Thailand using benchtop Fourier transform infrared (FT-IR) and hand-held near infrared (NIR) spectroscopy. A new rapid field-deployable technique using screen printed carbon electrodes (SPCE) with electrochemical detection was tested for the presence of heavy metals in turmeric teas and for routine screening of aflatoxins in pistachio nut samples. The control of agrochemical residues and natural contaminants in food remains a key concern for Member States. Response to Member States needs in this field included the development and validation of a multi-residue method over 140 pesticides and associated metabolites by liquid chromatography-mass spectrometry.

The FEPL coordinated and provided technical input into two coordinated research projects on food authenticity, involving approximately thirty countries.

Personnel from FEPL presented in many different fora including, four virtual international conferences, national conferences, technical webinars, media articles, and the FEPL was represented

in the scientific committees for two major international conferences on food safety and authenticity and organised a working group on food and agriculture applications in the IAEA's first major Technical Meeting on the use of Artificial Intelligence for Nuclear Technology and Applications. The FEPL contributed to international efforts to develop food fraud controls through participation in the UK's Food Authenticity Methodology Working Group, and the development of standards through the European Committee for Standardization (CEN) Technical Committee (460) 'Food Authenticity' Working Group 6, 'Stable isotope Analysis' of the European Commission.

The FEPL provided capacity building for Member States through technical management of fifteen national and three regional technical cooperation projects in 2021. Although many planned technology and knowledge transfer activities were disrupted due to the COVID-19 pandemic, human resource capability was enhanced in Member States through the training of more than 600 scientists, analytical chemists and laboratory personnel by means of virtual training workshops, the development of distance learning courses, and webinars implemented via networks such as the Red Analitica de Latinoamérica y el Caribe (RALACA). In addition, the FEPL hosted two interns and two PhD consultants.

The project, 'Enhancing Capacity in Member States for Rapid Response to Food Safety Incidents and Emergencies', funded by the government of Japan under the 'Peaceful Uses Initiative' (PUI), continued in 2021 with method development and four virtual training courses with over 160 participants. Additional PUI funding was obtained in November from the USA to develop a 'Food Authenticity Laboratory Network' and database framework for a global network of isotopes in food.

Thirteen papers with FEPL staff as co-authors were published in peer-reviewed scientific journals in 2021.

STAFF

Name	Title
Cannavan, Andrew	Laboratory Head (Jan – July)
Kelly, Simon	Food Safety Specialist (Jan – July) Acting Lab. Head (Aug – Dec)
Maestroni, Britt Marianna	Food Scientist
Nakaya, Shuichi	Food Safety Specialist
Mihailova, Alina	Analytical Chemist
Islam, Marivil	Laboratory Technician
Abrahim, Aiman	Laboratory Technician
Maxwell, Florence	Laboratory Technician
Faustmann, Aminata	Team Assistant
Permetov, Serik	Laboratory Attendant
Rezende, Sofia	PhD Consultant (food contaminants)
Migues, Ignacio	PhD Consultant (food authenticity)

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

The IAEA, including the Joint FAO/IAEA Centre for Nuclear Techniques in Food and Agriculture, helps its Member States to build capacity for food safety control through the technical cooperation programme and with upstream adaptive research at Seibersdorf and through coordinated research projects. FEPL has continued to focus on enhancing resilience in Member States and improving their abilities to respond rapidly to crises, such as the COVID-19 pandemic, that affect the food supply chain, human resource availability and the capacities of food control laboratories to implement regulatory testing, by developing and adapting rapid methods based on nuclear and complementary techniques to conduct 'tier 1' screening of foods. Furthermore, well-known hazards such as heavy metals and mycotoxins are regaining prominence in the context of climate change. These global challenges have highlighted the continued relevance of the project 'Enhancing Capacity in Member States for Rapid Response to Food Safety Incidents and Emergencies', which is funded by the government of Japan under the 'Peaceful Uses Initiative' mechanism and implemented by the Joint FAO/IAEA Centre's Food Safety and Control laboratory (FEPL). This project has enabled applied and adaptive research in FEPL to progress the development of more accessible and rapid analytical methods for the detection of food contaminants, adulterants and to verify the geographical and production origin of foods. The capacity to effectively carry out routine food surveillance and respond to incidents and situations compromising food safety is developed in Member States through enhancement of their knowledge and understanding of the potential underlying food safety and fraud food fraud problems and the tools available to control them. The application of analytical methods, that provide reliable food safety and authenticity data, enables government regulators, food safety authorities, enforcement agencies and the food sector to make science-based decisions to manage such situations and their associated risk to consumers. Effective technology and knowledge transfer were achieved through virtual training courses during the pandemic, the development of databases and other online resources, and facilitating laboratory networking.

Analytical method development in the FEPL encompasses both sophisticated techniques capable of providing essential confirmatory 'tier 2' information such as the identities and amount of food contaminants present or the probable geographical origin or production technique of a food product that allow follow-up actions to deal with safety incidents; and cost-effective, 'tier 1' screening, 'point of contact' field-based methods that can be deployed in the supply chain to provide rapid answers regarding the safety, quality or authenticity of food raw materials or products. A combination of these techniques through tier 1 screening and tier 2 confirmatory techniques, provides Member States with the options needed for their food control systems, both under normal circumstances and when the systems are challenged by crises or emergencies.

In 2021, eleven novel analytical methods and associated method protocols were developed in FEPL. Seven instrument or workflow operating procedures were prepared to standardize operations in FEPL and for use in training and technology transfer to Member States.

A selection of the main research activities and results are presented below.

Detection of food fraud

Food fraud may be defined as intentionally causing a mismatch between claims on food product labels and the actual characteristics of a food product. This deception is usually motivated by economic gain on the part of the perpetrator typically by using inferior and cheaper ingredients. Obvious examples are substituting food products, with added value claims, e.g. organic, free-range, wild-caught, natural, Protected Designation of Origin, etc, with similar but indiscernible products from a relatively inexpensive source, e.g. conventional, barn-fed, farmed, nature-identical, non-appellation, etc, respectively. Rather than completely substituting a food product it may be extended or fortified with an adulterant, or mixture of adulterants, that ostensibly maintain or enhance its characteristics whilst reducing production costs. It is at this point that the interface between food fraud and food safety is

often breached and this can also present a significant risk to human health. There have been many examples of this throughout history. In recent times, notable examples include melamine in milk powder, Sudan dyes in chilli powder and methanol in counterfeit spirits. The global occurrence of such incidents may cause negative impacts on international trade, reputational damage to companies or entire food sectors and, at worst, serious illness or fatalities, to consumers.

Rapid analytical methods to differentiate between Arabica and Robusta coffee

Coffee is one of the most widely traded commodities worldwide. The two main species of cultivated coffee are *Coffea arabica* L. (commonly known as Arabica), and *Coffea canephora* Pierre ex A. Froehner (commonly known as Robusta). Arabica coffee beans are highly valued among consumers for their superior smooth, mild and rich flavour and account for over 60% of global coffee production. Brazil, the world's biggest producer of the premium Arabica coffee beans, has seen its harvest significantly impacted by droughts and frosts in 2021, consequently raising the international market price of unroasted coffee beans. In 2021 the Covid-19 pandemic impacted the export supply chains from Vietnam, which also added to concerns over the global supplies of coffee. Vietnam is the world's second largest grower and a major producer of the Robusta coffee bean used in instant coffee and some espresso blends. The lockdown of Ho Chi Minh City severely affected Vietnam's ability to export its coffee beans. Consequently, wholesale Robusta bean prices rose by around 50% in 2021. The increase in the price of coffee has led to concerns that there will be an increased misrepresentation by unscrupulous traders to make economic gain by partially or wholly substituting Arabica beans with Robusta. Therefore, there is an urgent need for easily accessible analytical techniques that can be used to monitor the authenticity of Arabica coffee and screen samples in the supply chain.

Various spectroscopic and mass spectrometric techniques have been applied for the testing of coffee authenticity. There is, however, still a significant need to develop faster, cheaper and more efficient

Multispectral imaging MSI analysis of Arabica and Robusta coffee at FEPL.

methods to complement currently available techniques. Multispectral imaging (MSI) is an innovative and non-destructive technique that combines imaging and spectral technologies with advanced digital image analysis and machine learning. Using strobed light-emitting diode (LED) technology the MSI system combines measurements at 20 different wavelengths into a single high-resolution spectral image, permitting fast and accurate characterization of foods in terms of colour, surface chemistry, texture, shape, and size without touching the sample and often with no sample preparation. This technique can complement the various other analytical approaches that are being developed and tested at FEPL under the coordinated research project (CRP) D52040 "Field-deployable Analytical Methods to Assess the Authenticity, Safety and Quality of Food" and CRP D52042 "Implementation of Nuclear Techniques for Authentication of Foods with High-Value Labelling Claims (INTACT FOOD)" for transfer to the Member State laboratories.

In 2021 FEPL developed a rapid screening method for the differentiation between Arabica and Robusta coffee samples using the VideometerLab 4 MSI system. Overall, 10 types of Arabica and 8 types of Robusta roasted coffee, mostly of single origin (India, Uganda, Brazil, Mexico), were obtained for this study from a trusted supplier. No sample preparation was required for the MSI analysis. Spectral data

were obtained in 100% reflectance mode between 365 and 970 nm. Image data (e.g. bean width, length, area, roundness) were obtained by using the VideometerLab 'Blob tool'. Figure 1 shows representative MSI images of 100% Arabica (A), 100% Robusta (B) and a mixture of 50% Arabica (C, left) and 50% Robusta (C, right) beans with masked background. The respective images transformed using normalised canonical discriminant analysis (nCDA) are presented in Figure 1 A1-C1. nCDA allowed the separation of Arabica and Robusta coffee beans and demonstrated a potential for the two coffee species to be discriminated.
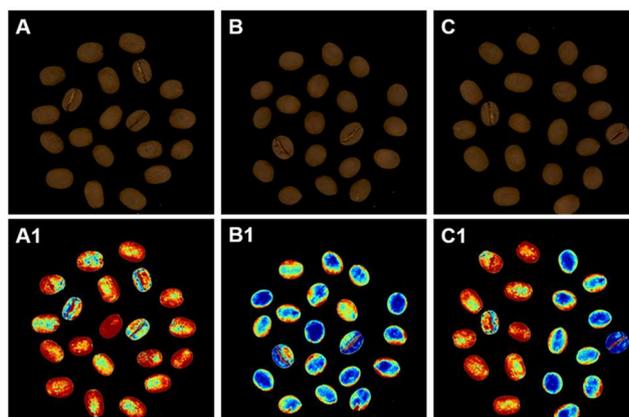
FIG. 1: Representative MSI images of 100% Arabica (A), 100% Robusta (B) and 50% Arabica and 50% Robusta (C) beans with masked background; and the respective nCDA images (A1-

Chemometric modelling was performed using the combined averaged and pre-processed spectral and morphological features of coffee beans in each Petri dish. First, an unsupervised principal component analysis (PCA) was carried out. PCA showed a tendency of samples to group according to the coffee species (Figure 2A). The goodness of fit (R2(cum)) and the predictive ability (Q2(cum)) of the PCA model were 0.909 and 0.834, respectively.
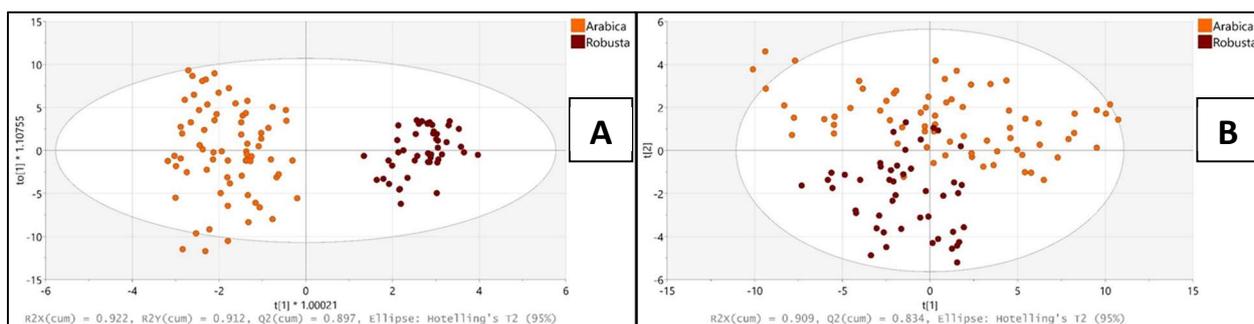
FIG. 2A: PCA model of the Arabica and Robusta coffee bean samples in the training dataset.
FIG. 2B: OPLS-DA model of the Arabica and Robusta coffee bean samples in the training dataset (in both models Arabica: n = 70, Robusta: n = 47).

The data were then subjected to supervised orthogonal partial least squares discriminant analysis (OPLS-DA). OPLS-DA allowed a clear differentiation between Arabica and Robusta coffee beans (Figure 5). The goodness of fit (R2X(cum), R2Y(cum)) and the predictive ability (Q2(cum)) of the OPLS-DA model were 0.922, 0.912, and 0.897, respectively. External validation of the OPLS-DA model was performed using the test dataset comprising samples that were not used in the construction of the model (Arabica: n = 35, Robusta: n = 23). The model achieved 100% correct classification of both Arabica and Robusta coffee species in the test dataset. The OPLS regression model was also able to successfully predict the level of adulteration of Arabica with Robusta. Consequently, FEPL has clearly demonstrated that MSI analysis has great potential as a relatively low-cost and rapid tier 1 screening tool for the detection of fraud issues related to the authenticity of Arabica coffee beans. The technology is also applicable to other commodities such as rice, herbs and spices and potentially to assess the identity, age and quality of meat and fish.

Development of a rapid low-cost isotope analysis of nitrate in fruit extracts to differentiate organic and conventional products

In 2021 FEPL has continued to work on methods to confirm the veracity of organic food labelling claims. The value of the global organic food and drink market reached nearly 100 billion US dollars in 2017 and is predicted to triple by 2025. The cultivation of organic crops is an important export market

for many developing countries. Organic produce is usually sold at a higher price compared to its conventional counterparts because of the additional labour costs and more . Organic crops are subject to routine safety monitoring, such as pesticide residue analysis, as part of national surveillance programmes, however there are no officially recognized end-product tests to verify the many aspects of organic production requirements, such as the unpermitted use of synthetic nitrate fertilizers. This is a major gap in the quality control of organic produce, since economic incentives to fraudulently mislabel conventionally cultivated crops as organic remain high. This has led to a significant incidence of fraud in recent years, at a cost of many millions of dollars.

The authenticity of organic products currently relies on enforcement of production standards through certification and inspection. The system depends on traceability through paper trails from farm to fork, and the potential exists for fraud at many stages in these supply chains, particularly with regard to imported produce. This creates an incentive for unscrupulous producers, distributors and retailers to mix, or completely substitute, organic and conventional produce for financial gain. A significant number of cases of fraud, where conventional produce was mislabelled and passed off as organic, have been reported in recent years worldwide. The issue concerns the whole agri-food sector, from small local farmers' markets to supermarket chains and global retailers. To support the integrity of the whole organic food and drink supply chain, it is therefore of paramount importance that, in addition to certification systems, the authenticity of organic foods can be independently assessed using analytical methods.

Organic food systems do not use synthetic nitrate fertilizers that possess characteristically high stable oxygen-18 isotope (δ^{18}O) values and relatively low stable nitrogen-15 isotope (δ^{15}N) values, compared to nitrate produced naturally by nitrifying bacteria in soil. This may be used as the basis to verify organic production labelling claims and implied fertilization practices. Consequently, the nitrogen and oxygen stable isotope composition (δ^{15}N, δ^{18}O) of nitrate in fruits and vegetables has been used successfully to differentiate organic from conventional food production practices. However, one major obstacle to rapid and low-cost compound-specific dual-isotope analyses of nitrate from plant or food extracts are the lengthy and costly techniques required to convert extracted nitrate into the gas used for measurement of the isotope signature, nitrous oxide gas (N_2O). Currently methods such as microbial denitrification or cadmium reduction have been used that require specialised microbiological maintenance of denitrifying bacteria or chemicals that are toxic and difficult and costly to dispose of, respectively. Rapid, low-cost methods are needed to facilitate nitrate isotope analyses of food products to support this aspect of organic food product certification and to verify the authenticity of production claims. FEPL, in collaboration with the IAEA's Isotope Hydrology Laboratory, developed a new titanium (III) reduction method providing a low-cost and rapid analytical method to facilitate the compound-specific δ^{15}N and δ^{18}O isotope analyses of nitrate and applied the method to the analysis of organic strawberries.

Authentic production samples of organic and conventionally cultivated strawberry fruits were obtained via a collaborating body, the SGF (Sure, Global, Fair, previously known as Schutzgemeinschaft der Fruchtsaft- Industrie e.V), a non-profit industrial association financed by more than 650 fruit juice companies from nearly 60 countries worldwide. Forty authentic production samples of organic (n=20) and conventionally produced strawberries (n=20) were obtained from Spain. One kilogram samples were supplied as frozen whole strawberries (not freeze-dried), with each sample coded to the location of the grower and whether the berries provided were certified organic or conventionally grown. The organic and conventional samples were from the Southern part of Province Huelva, Spain. Both types of strawberries were from cultivation under tunnel, where liquid fertilizer is added to the irrigation water, which is derived from surface or well water. The Ti(III)-based N and O stable isotope analyses of nitrate in strawberry extracts revealed clear stable isotopic differentiation between organic and conventional production operations, with mean δ^{18}O and δ^{15}N values of +18.3 ‰ (range: +15.9 to +20.7 ‰, SD: ±1.2 ‰) and +17.6 ‰ (range: +15.1 to +19.3 ‰, SD: ±1.2 ‰) versus +28.2 ‰ (range:

+21.0 to +37.3 ‰, SD: ± 4.5 ‰) and +14.9 ‰ (range: +10.3 to +22.3 ‰, SD: ±2.9 ‰), respectively (Figure 3). A students t-test revealed the mean δ^{18}O and δ^{15}N values for organic and conventional nitrate samples differed significantly from each other (p=0.05). The larger δ^{18}O difference in the nitrate between organic and conventional production of approximately 10 ‰ provided an unequivocal diagnostic differentiation between synthetic and organic fertilization practices. Notably, there was considerably less isotopic range and spread in the δ^{18}O values of nitrate extracted from the certified organic strawberry operations compared to conventional operations (Figure 3), suggesting that
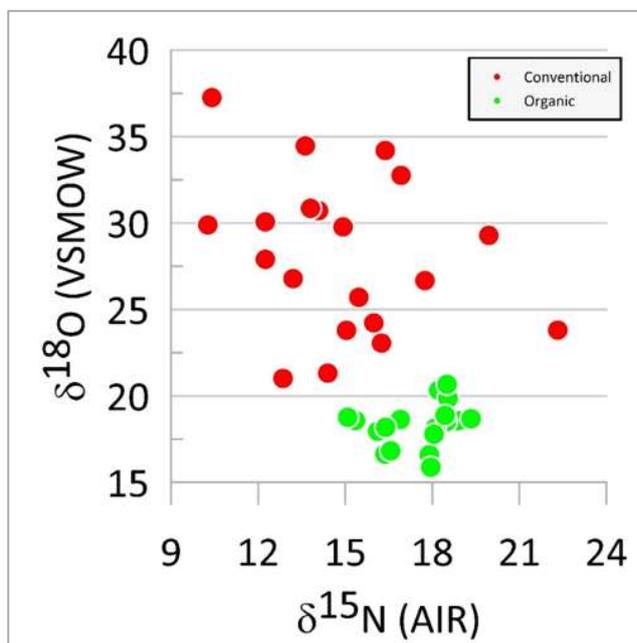
FIG. 3: *Stable isotope composition of nitrate (δ^{18}O and δ^{15}N measured as N_2O) in fresh strawberry extracts from conventional and certified organic fruit grower operations in Andalucía, Spain.*

certified organic production practices may be more consistent due to their biological-based fertilization practices. Because the δ^{18}O in nitrate derived from nitrification of organic fertilization is largely controlled by the oxygen isotopic composition of the (local) irrigation water used, there will likely be geographic differences in δ^{18}O amongst organic production operations over larger regions, which could, with further research, potentially be linked to known and predictable spatial oxygen isotope patterns to further evaluate the authenticity amongst organic growers at larger spatial scales. However, across our study area in Andalucía, the oxygen isotope range for weighted mean annual precipitation, likely reflected in irrigation water, was only around 1.5 ‰, and hence too small to explain the larger δ^{18}O differences observed for nitrate. The conventional strawberry operations, on the other hand, use a broader range of approaches and fertilizers, which was reflected in the greater isotopic

range and scatter for δ^{18}O and δ^{15}N difference between extractable nitrate between operational practices was smaller (2.7 ‰) and with overlap. Our results show the Ti(III) reduction method provides a new low-cost and rapid analytical method to facilitate compound-specific δ^{15}N and δ^{18}O isotope analyses of nitrate in selected fruit types, and likely other food products, for the purposes of assessing nitrate fertilization practices of organic versus conventional production claims and to support authenticity investigations. The ability to prepare and analyze 70 nitrate reference controls, and unknown samples (limited only by autosampler tray size) over 48 hours at low cost makes this method attractive for adoption by Member States. The total time to prepare 70 samples including the 12 calibration standards and 4 control standards was approximately 2 hours. The total cost of the reagents and vials was less than USD $10 per sample. Furthermore, potential exists to assess the geographic authenticity of certified organic fruits and vegetables by exploiting the highly predictable spatial patterns in δ^{18}O of irrigation waters, i.e. fruit or vegetable nitrate isotope maps (isoscapes).

Differentiation between organic and conventional fruit juices using bench-top NMR spectroscopy

Because of the multifaceted nature of organic agriculture, in order to comply with the production regulation, various analytical techniques have been investigated and applied over the past decade for the authentication of organic products. Undoubtedly, stable isotope analysis and elemental analysis have been the some of the most widely tested hypothesis driven techniques. Stable isotopes have proven to be good indicators of authenticity for both plant- and animal-derived food products; however, complete discrimination between organic and conventional foods is often not possible based solely on stable isotope analysis because of confounding factors such as the use of green

manures and legume crop rotation. It is generally accepted by researchers in this area that the authentication of organic food products is unlikely to be achieved by the measurement of a single or only a few selected markers. There is, therefore, a significant need for efficient, rapid and easily deployable methods for verifying the authenticity of organic food. The development of such methods continues to be a focus of FEPL work, in support of coordinated research projects D52040, 'Field-deployable analytical methods to assess the authenticity, safety and quality of food' and D52042, 'Implementation of Nuclear Techniques for AuthentiCaTion of Foods with High-Value Labelling Claims (INTACT Food)'.

In 2021 FEPL continued work on the development of methods for differentiating organic and conventional orange juices using a limited number of authentic samples from Mexico, supplied by the fruit juice industrial association, Sure-Global-Fair (SGF). The potential of benchtop proton nuclear magnetic resonance (^{1}H NMR) spectroscopy to differentiate the juices from organic and conventional production was investigated to build on work previously completed using FT-IR. ^{1}H NMR spectra were acquired using a Magritek Spinsolve 60 benchtop NMR spectrometer over the course of 5 minutes per sample. Spectral data were pre-processed using the following algorithms: zero filling, phase correction, baseline correction, referencing, and spectral alignment. Data were normalised, Pareto-scaled and subjected to chemometrics analysis.
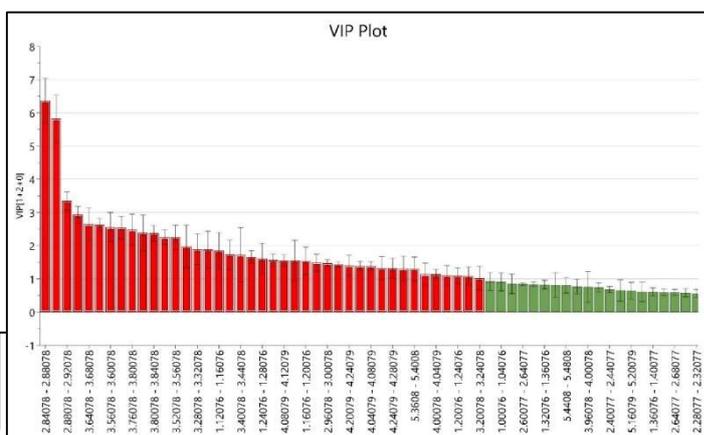
FIG. 5: *Variable Importance in Projection (VIP) plot of the OPLS-DA model of organic (n = 40) and conventional (n = 16) orange juice samples from Mexico.*

Principal component analysis (PCA), an unsupervised multivariate method, was used to assess the initial juice sample groupings. The PCA model (Figure 4A) showed the separation of the juice samples according to their production system, i.e. organic or conventional. The goodness of fit (R2X (cum)) and the predictability (Q2(cum)) values of the
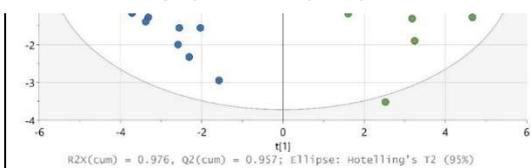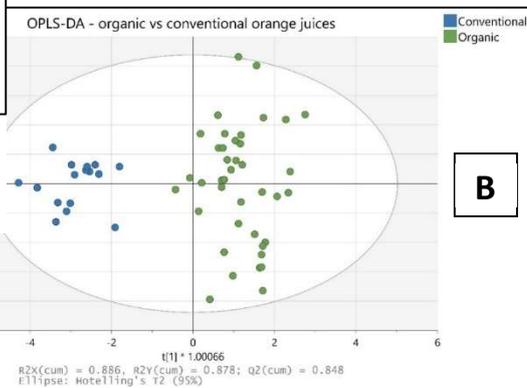
FIG. 4A: *PCA model of organic (n=40) and conventional (n=16) orange juice samples from Mexico.*
FIG. 4B: *OPLS-DA model of organic (n=40) and conventional (n=16) orange juice samples from Mexico.*

obtained PCA model were 0.976 and 0.957, respectively. The data were analysed using the supervised multivariate method, orthogonal projections to latent squares discriminant analysis (OPLS-DA), with seven-fold cross-validation. The OPLS-DA model (Figure 4B) successfully discriminated between the two groups, organic and conventional juice samples. The performance indicators of the OPLS-DA model, R2X (cum), R2Y(cum) and Q2(cum), were 0.886, 0.878 and 0.848, respectively. Using the limited number of samples available, an OPLS-DA model was constructed using a training dataset and subsequently used for the prediction of the production origin of the juice samples from a test dataset not used for model construction. The OPLS-DA demonstrated the reliability of the model and correctly predicted 100% of both organic and conventional orange juice samples. In order to better understand the underlying characteristics of the OPLS-DA model the major spectral features responsible for the differentiation of organic and conventional juices, were examined using the spectral data and the

Variable Importance in Projection (VIP) scores from the generated model (Figure 5). The major differences between organic and conventional orange juices were observed in the NMR spectral regions corresponding to citric acid (chemical shift: 2.88-2.84 ppm), glucose (3.90-3.70 ppm), fructose (4.12-4.04 ppm) and sucrose (5.40-5.36 ppm). The reliability of these models will be further tested through additional model validation, which will be performed upon receipt of more authentic organic and conventional orange juice samples from the SGF.

Geographical differentiation of Hom Mali rice cultivated in different regions of Thailand using benchtop FTIR-ATR and hand-held NIR spectroscopy

Rice is the most economically important crop in Thailand. Thai Hom Mali rice, also known as Thai Jasmine rice, is a non-glutinous fragrant rice, which is considered to be the highest quality rice in Thailand, valued for its unique aroma, soft texture and superb cooking quality. These qualities add retail value and make Thai Hom Mali rice more prone to adulteration and substitution. It is widely acknowledged that Thai Hom Mali rice from the north-eastern region of Thailand has a superior quality. This creates an incentive for unscrupulous producers, wholesalers and retailers to mislabel rice originating from other geographical regions and label it as the more valuable rice, cultivated in the Northeast of Thailand, for financial gain. The verification of the geographical origin of Thai Hom Mali rice is not readily undertaken in the rice supply chain, because the existing analytical approaches, e.g. stable isotope and trace element analysis (SITE), are relatively time-consuming and expensive from the analysis, equipment and maintenance point of view. Therefore, there is a significant requirement for rapid, low-cost and efficient 'tier 1' screening that can be used to monitor the authenticity of Thai Hom Mali rice and screen the samples in the supply chain before committing samples to 'tier 2' confirmatory SITE analysis.

Infrared (IR) spectroscopy is a rapid, non-destructive technique that requires little or no sample preparation and does not involve the use of chemicals or specialized laboratory facilities. Several studies have shown the potential of IR spectroscopy techniques, such as Fourier-transform infrared spectroscopy (FTIR) and near-infrared (NIR) spectroscopy, combined with chemometrics for the differentiation of geographical and botanical origin of various food products, e.g. cereals, honey, fruits, edible oils. IR spectroscopy offers an untargeted multi-analyte screening capability as well as low operational costs. This makes the technique suitable for authenticity screening, complementing the other analytical approaches that are being developed at FEPL under CRP D52040 "Field-deployable Analytical Methods to Assess the Authenticity, Safety and Quality of Food" and CRP D52042 "Implementation of Nuclear Techniques for Authentication of Foods with High-Value Labelling Claims" and transferred to the Member State laboratories.

Two IR spectroscopy techniques were investigated in FEPL, a benchtop FTIR fitted with an attenuated total reflectance (ATR) accessory, for simple and easy sampling of liquid and solids, and a low cost (< 1,000 USD) hand-held NIR, coupled with multivariate statistics (orthogonal projections to latent structures discriminant analysis or OPLS-DA), for the differentiation of Hom Mali rice from the north-eastern and northern regions of Thailand. A total of 170 Thai Hom Mali rice samples, supplied by the Thailand Institute of Nuclear Technology, were used for this study. Samples were collected from the northern and north-eastern regions of Thailand during two production years: 2018 and 2019. FTIR spectra were collected in reflectance mode between 4000 and 450 cm^{-1} at a resolution of 1 cm^{-1} using a benchtop FTIR-ATR. Examples spectra are shown in Figure 6A. NIR spectra (Figure 6B) were collected between 740 nm and 1070 nm at a resolution of 1 nm using a hand-held NIR spectrometer (Consumer Physics SCiO™). The average of replicate scans was pre-processed using multiplicative scatter correction (MSC) and the 1st derivative functions. The full dataset from each production year was divided in randomised order into a training set (n = 47 (2018); n = 67 (2019)) and a test set (n = 23 (2018); n = 33 (2019)). OPLS-DA with seven-fold cross-validation was used to build the discriminative models for the differentiation of northern and north-eastern region in the training dataset. The performance of the models was assessed using the goodness of fit ($R2$) and predictability ($Q2$) values. The OPLS-DA models, built using the training dataset,

FIG. 6. Representative benchtop FTIR-ATR (A) and hand-held NIR (B) spectra of Thai Hom Mali rice. Blue spectrum - north-eastern region; red spectrum - northern region.

were used to predict the geographical origin of the samples from the test dataset. The predictive ability of the models was assessed using the correct classification rate of samples from each region. For additional validation, the OPLS-DA model, obtained using the 2019 dataset (largest dataset), was used to predict the samples from the northern region (the only region with common provinces in both years) from the 2018 dataset. In addition, the effect of sample preparation (ground samples vs no sample preparation) on the performance of OPLS-DA models, obtained using NIR spectral data, was assessed.

The major differences in the FTIR absorbance bands between the samples from the north-eastern and northern regions were observed around 1030 and 1100 cm^{-1} (C-O stretching), which is associated with the presence of polysaccharides, around 1540 cm^{-1} (N-H bend, C-N stretch) and 1640 cm^{-1} (C=O stretch), associated with the presence of proteins, and around 2930 cm^{-1} (CH_2 symmetric stretch), associated with the presence of lipids. OPLS-DA allowed clear differentiation between the rice samples from the northern and north-eastern regions in both production years (Figure 6). The goodness of fit ($R2$) and the predictive ability ($Q2$) of the OPLS-DA model are shown in Table 1. External model validation was performed using the test dataset comprising of samples that were not used in the construction of the model. The correct classification rate of samples from the test dataset was 100% and 96.97% in 2018 and 2019, respectively.

Table 1. Benchtop FTIR-ATR: goodness of fit and predictive ability of OPLS-DA models for the 2018 and 2019 datasets.

Spectroscopic technique	Year	N (train. set)	N (test set)	R2X (cum)	R2Y (cum)	Q2 (cum)	Correct classification rate of the test set, %		
							Northeast	North	Total
Benchtop FTIR-ATR	2018	47	23	0.919	0.981	0.776	100	100	**100**
	2019	67	33	0.637	0.929	0.477	96.65	100	**96.97**
Hand-held NIR	2018	47	23	0.992	0.771	0.409	94.12	66.67	**86.96**
	2019	67	33	0.969	0.734	0.488	91.30	70.00	**84.85**

N – number of samples; train. set – training set.

The predictive ability of the OPLS-DA model, generated using the larger dataset of 2019, was challenged by using the samples of 2018 as "unknowns". Due to the fact that the samples from north-eastern region came from different provinces in 2018 and 2019, only samples from the North (common provinces in both years) were used for this additional model validation. The OPLS-DA model was able to correctly predict 100% of the "unknown" samples from the North from 2018.
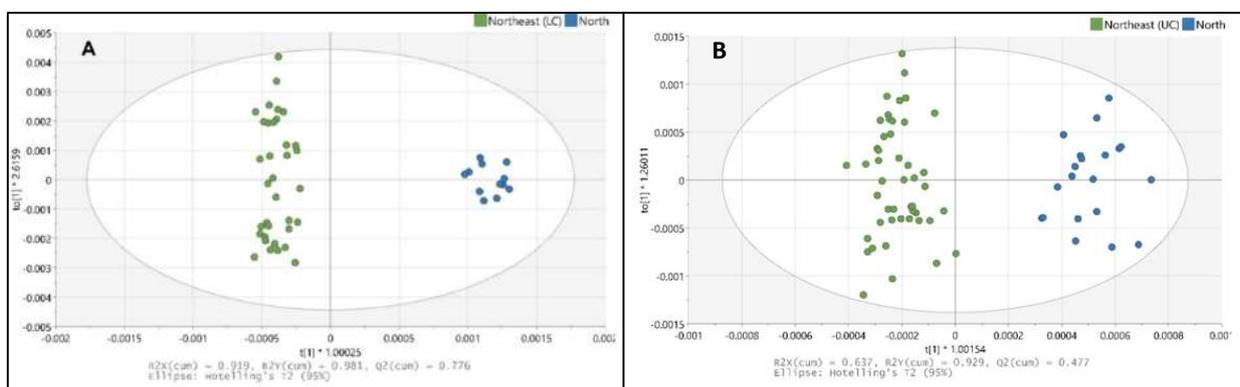
FIG. 6: *OPLS-DA models of the training dataset analysed with FTIR-ATR: year 2018 (A) and 2019 (B).*

The NIR spectra, obtained using a hand-held NIR spectrometer, also allowed differentiation between the rice samples from the northern and north-eastern regions in both production years. The goodness of fit (R2) and the predictive ability (Q2) of the OPLS-DA model (Figure 3) constructed using NIR data are shown in Table 1. External model validation was performed using the test dataset comprising samples that were not used in the construction of the model. The correct classification rates of samples from the test dataset were 86.96% and 84.85% in 2018 and 2019, respectively. The predictive ability of the OPLS-DA model, generated using the larger dataset of 2019, was challenged by using the samples of 2018 as "unknowns". The OPLS-DA model was able to predict 100% of the "unknown" samples from the North from 2018.

In addition to ground rice (n=100), whole rice grain samples (n=100) from 2019 were analysed by hand-held NIR, and the effect of both sample preparation techniques on the performance of OPLS-DA models was assessed. No decrease in the performance of the model was observed for whole grain rice samples as compared to ground rice powder. The correct classification rates of samples from the test set were 87.88% and 84.85% for whole grain and ground rice samples, respectively. These results demonstrate that in addition to its low cost, small size and ease of use, the hand-held NIR spectrometer is suitable for the analysis of dehulled polished rice without the need of any sample preparation, which makes the approach very rapid and straightforward.
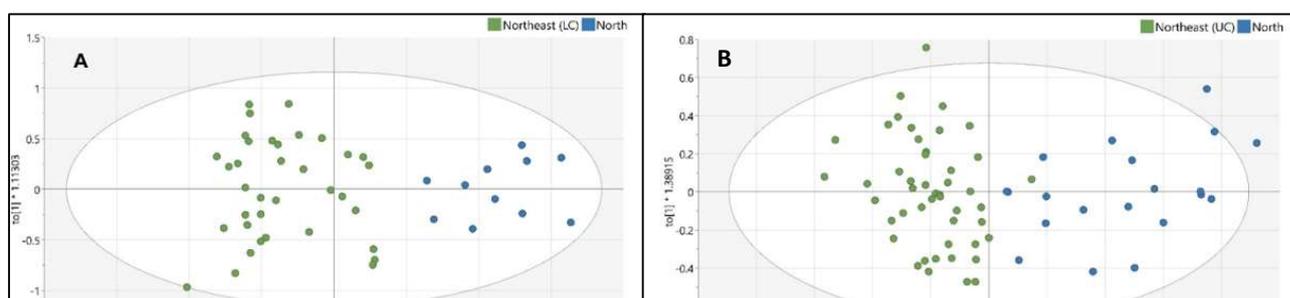
This study demonstrated that benchtop FTIR-ATR and hand-held NIR spectroscopy, combined with OPLS-DA, are promising analytical tools for geographical differentiation of Thai Hom Mali rice. This opens up the possibility for these types of rapid spectroscopy approaches to be used for cost-effective sample screening at a farm or retail level before committing to more sophisticated and time-consuming SITE techniques for confirmatory or orthogonal analysis by other complementary techniques.

Coordinated research on food authenticity

In 2021, FEPL coordinated and provided technical input into two coordinated research projects (CRPs) in the field of food authenticity.

Field deployable analytical methods to assess the authenticity, safety and quality of food (D52040)

This project started in March 2017 and held its third research coordination meeting (RCM) to exploit and adapt bench-top, portable and hand-held nuclear and molecular spectroscopic screening technologies, for front-line food fraud detection, as an on-line event from the 07 – 18 June 2021, due to Covid-19 travel restrictions. Despite the challenges of the Covid-19 pandemic and the detrimental effects of lock-down periods on access to laboratories and the opportunities for authentic sample collection, good progress has been made by the project consortium. Major achievements include 1) the establishment of the IAEA Shared Analytical Data Library Upload Tool, established through a technical contract with the Walloon Agricultural Research Centre (Belgium) as a repository for spectra for authentic vegetable oil and milk powder samples gathered through the various rapid screening techniques (near infrared, mid infrared, Raman, nuclear magnetic resonance); 2) the distribution of the sealed calibration units of vegetable oil and milk powder to ensure inter-comparability of spectra between different laboratories and equipment; 3) the finalization of the open-access multivariate statistics Add-in For Excel (CAFE) software and E-Learning package; and 4) significant scientific output as evidenced by 10 papers published in peer-reviewed journals since the second RCM in 2019.

Implementation of Nuclear Techniques for AuthentiCaTion of Foods with High-Value Labelling Claims (INTACT Food) (D52042)

This project started in 2019 and the second RCM for the 5-year project was held as an on-line event from the 01 – 12 November 2021. Good progress has been made despite the Covid-19 work and travel restrictions. The focus of the next phase of the project is to ensure that any delays due to the Covid-19 pandemic can be rectified as soon as practicable to ensure sufficient sampling, consistency of methods and data quality between participants so that the ultimate goal of generating a sustainable database can be achieved. To this end the addition of the new zero-cost Agreement Holder, NIST, and their contribution of an open access database with in-built chemometric and artificial intelligence interpretation tools is a major step forward for the CRP. Furthermore, supplementary funding for the project was received from the U.S. State department. This funding was provided to support an additional five research contracts from Latin America and the Caribbean (LAC) and observer presentations from Argentina and Chile were invited in order to present their ideas for joining the project. The new Argentinean project will be focused on important export products beef and citrus juices and the new Chilean project will be focused on high value export products shellfish, truffles and other products. The USA funding will also be used to support the formation of a food authenticity laboratory network in the LAC region; and finally, to support the construction of the open-access "Global Network of Isotope in Food" (GNIF) database.

Control of residues and contaminants in food

Screening and Confirmation of Aflatoxins in Food

Climate change has led to an increased risk of aflatoxin contamination in food products in regions of the world that were previously not exposed to such food contaminants. This is a direct consequence

of modified meteorological conditions, with persistent humid conditions and increased average temperatures. Ingestion of even low amounts of aflatoxins can generate severe adverse effects in humans, so their levels in food commodities must be strictly regulated. It is, therefore, of paramount importance to be able to quickly identify contaminated commodities to protect the health of consumers. A collaborative study between the FEPL and the University of Oviedo, Spain, has started, involving the preparation of pistachio sample extracts and their analysis by the laboratory in Oviedo using a rapid screen-printed carbon electrode (SPCE) as a 'tier 1' method and by FEPL using ultra high-performance liquid chromatography coupled to tandem mass spectrometry (LC-MSMS), as a confirmatory 'tier 2' method.

Development of a competitive immunoassay for total aflatoxins using screen-printed carbon electrodes

Screen printing technology is a well-developed method widely used to fabricate disposable and economical electrochemical sensors. Screen printed carbon electrodes (SPCEs) can provide highly reproducible, sensitive, and cost-effective detection methods. Their adaptability and ease of modification are of great importance and allow for detection of specific targets, such as total aflatoxin in pistachio and peanut butter. The Nanobioanalysis Group of the Department of Physical and Analytical Chemistry, University of Oviedo developed an immunosensor for the electrochemical detection of total aflatoxins (AFB1, AFB2, AFG1, AFG2) under a technical contract financed by the IAEA technical cooperation project VIE5022. The scheme of the competitive immunoassay is shown in Figure 8.
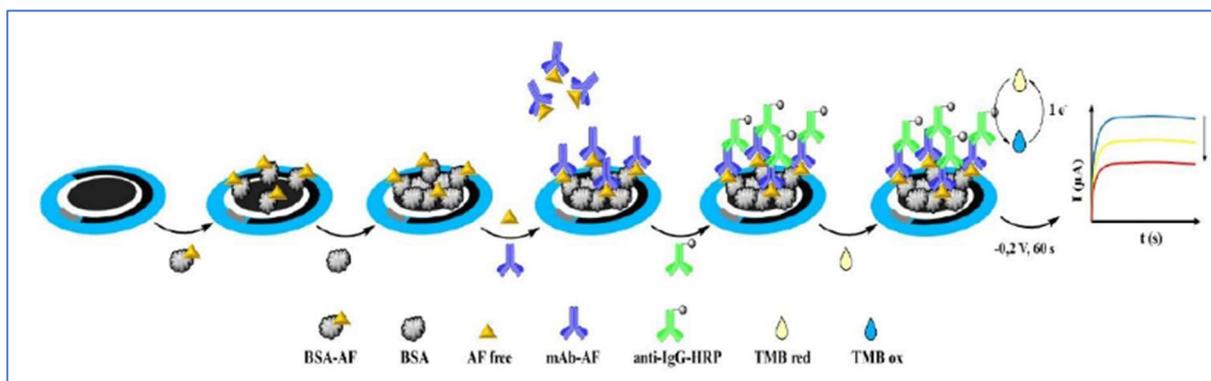
FIG. 8: Scheme of the competitive immunoassay for total aflatoxins performed on the surface of screen-printed carbon electrodes (SPCEs).

The bovine serum albumin (BSA)-labelled antigen, in this case aflatoxins (BSA-AF) immobilized on the SPCE surface, and the free AF analyte compete for the specific monoclonal antibody (mAb-AF). The horse radish peroxidase (HRP) enzyme is coupled to the electrode through a polyclonal secondary antibody (anti-IgG-HRP). After that, the added 3,3',5,5'-Tetramethylbenzidine (TMB) reagent is enzymatically oxidized by the HRP molecules. The oxidized TMB is reduced on the surface of the SPCE by applying a constant potential of −0.2 V for 60 seconds, producing an associated catalytic current (analytical signal) that is proportional to the initial AF amount. The developed SPCE immunosensor is simple, rapid, sensitive and makes use of low-cost instrumentation. The excellent performance observed in pistachio samples together with a detection limit below the Codex legal maximum level of total AFs, makes this approach a promising tool for the determination of total aflatoxins at the point-of-need, using a smartphone interface. This approach also offers a rapid development stage for emerging challenges, e.g. other food contaminants, as the sensor development is simple and straightforward.

Method optimization for determination of aflatoxins in pistachio samples by LC-MSMS

Three prospective LC-MSMS methods were evaluated for the determination of aflatoxins in pistachio samples. Samples of blank organic pistachios from a supermarket were homogenised in an automatic pestle and mortar mill using liquid nitrogen. Individual aliquots of pistachio paste were fortified and analysed to compare the methods, which differed in the extraction solvent used, the use of C18 clean-up columns and the fractionation solvent used and the amount. The aflatoxins were individually analysed, and the method that provided the best recovery values for all the aflatoxins (B1, B2, G1 and G2) and had the lowest number of analytical steps was selected. In the selected method, pistachio samples are extracted with methanol in the presence of a saturating salt, with thorough mixing and centrifugation steps. The upper layer of organic solvent is diluted with Milli Q water and applied to an immunoaffinity column, washed with water and eluted in methanol. The filtered extract is injected into a LC-MS/MS instrument programmed for the MRM transitions shown in Table 2. The method is currently being validated, and sample extracts will be prepared and sent to the Oviedo laboratory for the intercomparison study using two different detection technologies. Future work will include the incorporation of additional mycotoxins and the validation of the method for peanut samples

Table 2. Optimized MRM transitions for the detection of aflatoxins by LC-MS/MS.

Name	RT (min)	Polarity	Transition1 (*m/z*)	CE	Transition2 (*m/z*)	CE	Transition3 (*m/z*)	CE
Aflatoxin B1	4.67	+	313.1>241.1	-28.0	313.1>285.1	-34.0	313.1>269.1	-22.0
Aflatoxin B2	4.28	+	315.1>259.2	-22.0	315.1>287.2	-22.0	315.1>243	-20.0
Aflatoxin G1	3.81	+	329.1>243.1	-20.0	329.1>311.2	-26.0	329.1>200.2	-24.0
Aflatoxin G2	3.40	+	331.1>313.05	-23.0	331.1>245.2	-32.0	331.1>189.05	-41.0

Dissemination of Research Results

The results of the research and the methods developed or adapted and validated in the FEPL are typically 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.

In 2021, the in-person planned events were either postponed or replaced with virtual events and/or distance learning due to travel restrictions associated with the Covid-19 pandemic.

Conferences & Webinars

Food Integrity Online Conference 19-23 April 2021: Panel discussion on the Biggest Challenges and Solutions in Food Fraud and Analysis. Due to the Covid-19 pandemic and restrictions on global travel the conference organisers "New Food" launched the Food Integrity 2021 conference as a fully virtual, interactive five-day event. The conference explored the global food safety and authenticity challenges that the food industry is facing, with leaders from the food and beverage sector sharing and debating the issues, the lessons learned and possible solutions. Over 75 expert speakers, 1000 participants from across the globe, and exhibitors from industry attended the virtual event. During the conference, on the 21st April 2021, Mr Simon Kelly participated in a panel discussion on "The Biggest Challenges and Solutions in Food Fraud and Analysis" moderated by Prof Chris Elliott, Queen's University Belfast (QUB), UK. Mr Kelly explained that the ultimate goal is to protect consumers from food fraud and any of its unintended consequences that often impact on food safety and human health, especially in developing countries, as well as helping member states protect and promote their food products and facilitate international trade. Mr Kelly went on to describe how FEPL addressed this issue through an international CRP (D52040) that started 4 years ago bringing together researchers and instrument

suppliers from both developed and developing economies, to work on vegetable oil and milk authenticity using hand-held, field-portable and bench top systems based on molecular spectroscopic techniques that provide end-users with rapid, low-cost, and easy-to-use "screening" techniques, thereby increasing accessibility for testing to LMIICs.

UK Government Chemist Online Conference: Safe Food for Tomorrow's World, 23 and 24 June 2021.
The UK Government Chemist Conference was originally scheduled to take place in June 2020 but was postponed due to the COVID-19 pandemic. The programme included both national and international perspectives on food safety and food integrity and the use of science for improved consumer protection and developing consumer trust in the manufacturing, packaging, retailing and testing of food. The conference was aimed at policy makers, regulators, enforcement agencies, researchers and industry technical officers. Mr Simon Kelly was invited to give a pre-recorded lecture on 'Nuclear and complementary field-deployable technologies to build food authenticity capability' and then participate in live questions and answers during the session 'Novel solutions for food authenticity and sustainability'. Mr Kelly presented an overview of the activities of the FAO/IAEA Joint Centre's FEPL and reported specifically on some successful outcomes of the CRP D52040 'Field-deployable analytical methods to assess the authenticity, safety and quality of food'. The presentation was well received and gave rise to several questions from the meeting participants, relating mainly to the use and reliability of hand-held screening devices and the databases and multivariate analysis needed to deploy them effectively.

Latin American Risk Assessment Symposium (LARAS 2021) 14, 18, 19 and 25 October 2021.

The second Risk Assessment Symposium for Latin America and the Caribbean (LARAS 2021), co-sponsored by the IAEA among other international organizations, was held virtually, jointly hosted by the Chilean Agency for Food Safety and Quality (ACHIPIA) and the German Federal Institute for Risk Assessment (BfR) in 2021. The symposium aimed to contribute to risk assessment as an official approach to food safety, rooted in cooperation, state-of-the-art science, and trust. The FEPL actively contributed to the agenda, providing two speakers and moderating the event on 18th October. The events were broadcast simultaneously by two virtual platforms (Zoom and YouTube). On 14th October, the inauguration and risk assessment in food integrity day, Mr Andrew Cannavan, Head of the Food Safety and Control Section, contributed an opening speech emphasising that the FAO/IAEA Centre is a strong partner in relation to the scope of LARAS. At the same event Mr Simon Kelly, acting Head of the FEPL, gave a keynote speech on the general and methodological framework for the use of nuclear technologies, "Stable isotope and trace element (SITE) profiling". On 18th October Ms Britt Maestroni acted as a moderator for the event. The theme of the day was on novel risks and foods. After the event, Ms Nuri Gras, head of ACHIPIA, Chile, commented, "LARAS 2021 was an extremely enriching experience in which we were able to see the importance of working together in a coordinated way to strengthen science in the food area, specifically risk assessment in the countries of the Latin America and the Caribbean region". The videos of LARAS 2021 can be accessed online https://www.fao.org/americas/eventos/ver/en/c/1442636/.

CAPACITY BUILDING

The FEPL provided the main technical management for fifteen national and three regional technical cooperation projects in 2021. Many planned technology and knowledge transfer activities were disrupted due to the ongoing pandemic. However, support was maintained through the provision of advice and guidance, procurement, and in some cases through the development of alternative training methods, such as virtual training courses and webinars, greatly assisted by the laboratory networks such as RALACA. This enabled the training of more than 600 Member State scientists, technicians and regulators.

On-line training provided under the Peaceful Uses Initiative project, "Enhancing Capacity in Member States for Rapid Response to Food Safety Incidents and Emergencies"

Detection and Control of Organic Contaminants in Food

Due to the continued impact on travel and quarantine restrictions related to the COVID-19 pandemic, this follow-up in-person laboratory-based course at Seibersdorf, scheduled for 7-11 June 2021, was delivered as an on-line event. The training imparted knowledge on the general principles and application of selected analytical techniques and to provide participants with the ability to transfer the techniques and methods to their own environment and infrastructure. Several external experts and FEPL collaborators helped to provide expertise and training materials for this course in the form of video presentations. The methods and laboratory demonstration videos involved the use of equipment and instrumentation from a variety of different manufacturers, and various software applications for instrument operation, data acquisition and processing and statistical analysis. The course also included an overview of analytical instrumentation for targeted detection and control of organic contaminants in food such as LC and GC tandem mass spectrometry instruments, and a section on stable isotope dilution assays as well as a section on experimental design for LC-MS and GC-MS applications. The complete training course is available online at the IAEA Nucleus portal (Virtual Laboratory Training on the Detection and Control of Organic Contaminants in Food - Thumbnails (iaea.org). The participants of the training course had one week to access the video materials, before being invited to participate in live question and answer (Q&A) sessions, to clarify any details in the training materials and receive advice from the experts and collaborators. Although there were limited opportunities for interaction with the FEPL staff or the external experts providing demonstrations, the participants rated the Q&A sessions as very valuable.

Use of Profiling/Fingerprinting Techniques to Determine Food Origin and Verify Food Authenticity
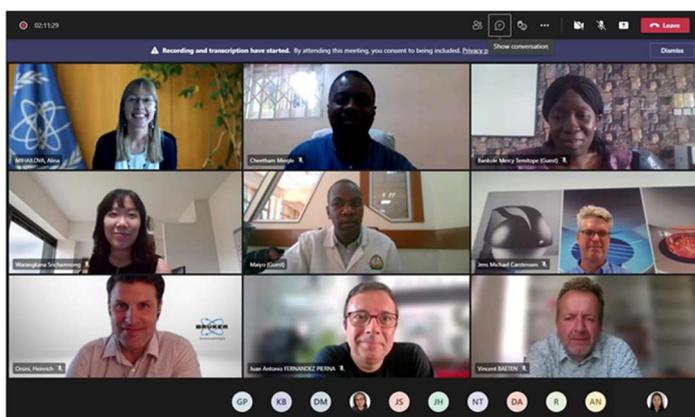
Ms Alina Mihailova moderating the Q&A session on infrared spectroscopy and multispectral imaging.

This second virtual training course under the PUI was held between 16-27 August 2021 and attended by 31 scientists from institutes in 24 countries: Australia, Bolivia, Chile, Denmark, Ecuador, Egypt, Ghana, Indonesia, Jordan, Kenia, Nigeria, Myanmar, Oman, Pakistan, Qatar, Russian Federation, Saudi Arabia, Senegal, Seychelles, Singapore, Sri Lanka, Thailand, Tajikistan and Uruguay. The training was designed to enhance the capabilities of laboratory personnel in Member State institutions in the application of rapid, untargeted screening methods, enabling them to respond to food safety-related incidents and emergencies and to improve their food control systems. The following techniques were covered in the course; benchtop nuclear magnetic resonance (NMR) spectroscopy; ion mobility spectrometry (IMS); Fourier transform infrared (FTIR) spectroscopy, including attenuated total reflectance (FTIR-ATR); Near-infrared spectroscopy (NIR); Multi-spectral imaging (MSI); and spectral data processing and chemometrics to enable model building and interpretation of the data and authenticity of testes samples. The virtual training employed recorded lectures, video presentations of laboratory procedures (sample preparation, instrumental analysis, data processing, multivariate statistics) and 'live' online question-and-answer sessions. In addition, examples of standard operating procedures and method protocols were provided to course participants, to foster adoption of the demonstrated methods in their own laboratories. Course materials were made available to participants via the NUCLEUS SharePoint site. The training course was very well received by the participants and anonymously evaluated by them through an on-line feedback questionnaire. Of the

participants who completed the questionnaire, 94.1% reported that 75-100% of course material was useful to their work, and all respondents indicated that the information provided during the course would be implemented in their activities/laboratory work at their home institution.

The Use of Stable Isotope and Trace Element (SITE) Profiling to Determine Food Origin and Verify Food Authenticity

The third training course was delivered through blended E-learning via the IAEA Nucleus SharePoint platform and the CLP4NET portal from 11 to 22 October 2021. The purpose of the training was to strengthen Member States' surveillance and research laboratory capacities in using the nuclear techniques, isotope ratio mass spectrometry (IRMS) and energy dispersive X-ray fluorescence (EDXRF) spectrometry to verify labelling claims related to the origin and authenticity of food products. The training also included an introduction to multivariate analysis of stable isotope and trace element (SITE) data using the Chemometric Add-in for Excel (CAFE) software, which included principal component analysis (PCA), 'one-class' soft independent modelling by class analogy (SIMCA) and partial least squares discriminant analysis (PLSDA). This training was designed to underpin effective control measures to protect consumers from fraud, including any associated unintended safety issues, mitigate the disruptive impact of emergencies affecting the food chain, and minimize disruption to trade in agricultural commodities. 108 scientists registered for the on-line training course from 31 countries; Albania, Algeria, Argentina, Australia, Austria, Brunei Darussalam, Burkina Faso, Chile, Costa Rica, Egypt, India, Indonesia, Italy, Lebanon, Mongolia, Morocco, Myanmar, Oman, Pakistan, Paraguay, Philippines, Qatar, Romania, Russian Federation, Slovenia, Sri Lanka, Sudan, Thailand, Turkey, United States of America and Uruguay. The 10-day course included pre-recorded theoretical lectures that could be accessed on-demand, videos of practical laboratory procedures such as sample preparation and analysis, multivariate assignments with CAFE software and multiple live question-and-answer (Q&A), sessions with the expert trainers, for the convenience of participants in different time-zones. Assessments of the knowledge gained by participants was made through on-line multiple-choice questionnaires in Google forms and assignments in Excel covering the multivariate analysis techniques. The training was generally well received by the participants and anonymously assessed by 46 respondents with 53% rating the training as "excellent" or "very good". Importantly, 91% of respondents to the questionnaire would recommend the training to other colleagues and 98% would like to be informed of future similar training events under the PUI project. The content from the Training course can be accessed here by registered IAEA Nucleus account holders Training Course on the Use of SITE Profiling to Determine Food Origin and Verify Food Authenticity - All Documents (iaea.org) and Course: Introduction to X Ray Emission Spectrometry (iaea.org).

RALACA Laboratory Network

The Analytical Network of Latin America and the Caribbean (RALACA) is a non-profit network of

RALACA/EFSA post-congress course on data sharing, hosted on 21 May through the LAPRW web platform.

laboratories and associated institutions, that aims to improve technical capabilities and encourage cooperation and communication between laboratories belonging to LAC region. During the COVID-19 pandemic RALACA continued supporting the development and improvement of the capacities necessary to guarantee food safety and a sustainable agricultural environment in the LAC Region by fostering communication and sharing of data and analytical information. In particular, in the period January-May 2021, RALACA hosted a

cost-free, 4 day distance learning course, implemented by the members of the ACADEMIA RALACA Committee, on "Introduction to residue and chemical contaminants analysis". More than 200 participants attended the training and valued the training course as most relevant to their jobs. ACADEMIA RALACA was formed in 2020 as a new Committee and is planning to organize further training courses for the Latin American and the Caribbean (LAC) region based on specific request by regional laboratories. RALACA also hosted a webinar on 9 February on "Veterinary drugs in food of animal origin: from single-class to multi-residue LC-MS analysis. How to improve reproducibility and recovery of some analytes" prepared by Dr. Serena Lazzaro from Italy. On 18th May 2021, on the occasion of the 8th Latin American Pesticide Residue Congress, RALACA hosted its 4th general meeting, and prepared a generic presentation on the outputs of RALACA during 2019-2021. On 21 May, RALACA, in conjunction with EFSA, delivered a free course on the topic, data sharing. More than 120 participants from the LAC region attended the course and showed a deep interest through a series of interactions in the Q&A session. The FEPL helped with the technical organization of the course and preparing the agenda. The RALACA board would like to encourage the active participation of all RALACA Institutions in the work of the different Committees and invite all Institutions to host online presentations of their own activities and infrastructure. Please contact the RALACA board for further information (ralacaboard@gmail.com).

Advice and Information Exchange

In 2021, staff of the FEPL were members of the scientific or organising committees of two international conferences. Mr. Andrew Cannavan served on the committee for EuroResidue IX: Current issues and emerging trends in residue control, which was planned to be held in The Netherlands in May 2020 but was postponed due to COVID-19 restrictions. The conference will now be merged with the 8th International symposium on Hormone and Veterinary Drug Residue Analysis and is planned for May 2022, venue to be decided. Mr. Cannavan also serves on the scientific committee for the ASSET 2022 Belfast Summit on Global Food Integrity, and on the Advisory Panel for the ASSET 2021 Food Industry Forum, UK. Mr. Simon Kelly participates in the European Commission's CEN Technical Committee (460) 'Food Authenticity' Working Group 6, 'Stable isotope Analysis' and the UK's Food Authenticity Methodology Working Group. Ms. Britt Maestroni serves on the Board and provides advice through various committees of the RALACA network.

Fellowships, Scientific Visitors and Interns

Name	Country	Status	Duration	Topic
Liebisch, Beatrix	Austria	Intern	12 months	Analytical methods for food control
Gola, Vamsi	India	Intern	12 months	

PUBLICATIONS

M^CVEY, C., ELLIOTT, C.T., CANNAVAN, A., KELLY, S.D., PETCHKONGKAEW, A. AND HAUGHEY, S.A. (2021). Portable spectroscopy for high throughput food authenticity screening: Advancements in technology and integration into digital traceability systems. Trends in Food Science and Technology,118, 777-790, https://doi.org/10.1016/j.tifs.2021.11.003.

YONG, C-H., MUHAMMAD, S.A., AZIZ, F.A., NG, J-S., NASIR, F.I., ADENAN, M.N.H., MOOSA, S., ABDULLAH, S.N.A., SHARIF, Z., ISMAIL, F., KELLY, S.D., CANNAVAN, A. AND SEOW, E-K. (2022). Detection of adulteration activities in edible bird's nest using untargeted ^{1}H-NMR metabolomics with chemometrics. Food Control, 132,108542. (Published online September 2021).

SRINUTTRAKUL, W., MIHAILOVA, A., ISLAM M.D., LIEBISCH, B., MAXWELL, F., KELLY, S.D., AND CANNAVAN, A. (2021). Geographical Differentiation of Hom Mali rice cultivated in different regions of Thailand using FTIR-ATR and NIR spectroscopy. Foods, 10, 1951, https://doi.org/10.3390/foods10081951.

YONG, C.-H., MUHAMMAD, S.A, AZIZ, F.A., NASIR, F.I., MUSTAFA, M.Z., IBRAHIM, B., KELLY, S.D., CANNAVAN, A. AND SEOW, E.-K. (2021). Detecting adulteration of stingless bee honey using untargeted ^{1}H-NMR metabolomics with chemometrics. Food Chemistry, https://doi.org/10.1016/j.foodchem.2021.130808.

KELLY, S., ABRAHIM, A., RINKE, P. AND CANNAVAN, A. (2021). Detection of exogenous sugars in pineapple juice using compound-specific stable hydrogen isotope analysis. Npj Science of Food, DOI: 10.1038/s41538-021-00092-5.

MIHAILOVA, A., KELLY, S.D., CHEVALLIER, O.P., ELLIOTT, C.T., MAESTRONI, B.M. AND CANNAVAN, A. (2021). High-resolution mass spectrometry-based metabolomics for the discrimination between organic and conventional crops: A review. Trends in Food Science and Technology, 110, 142-154.

HAYAR, S., ZEITOUN, R. AND MAESTRONI, B. (2021). Validation of a rapid multiresidue method for the determination of pesticide residues in vine leaves, comparison of the results according to different conservation methods. Molecules, 26, 1176.

JAMWAL, R., AMIT, KUMARI, S., KELLY, S., CANNAVAN, A. AND SINGH, D.K. (2021). Non-targeted fingerprinting approach for rapid quantification of mustard oil adulteration with linseed oil: an economically motivated adulteration. Vibrational Spectroscopy 113, 103226.

JAMWAL, R., AMIT, KUMARI, S., SHARMA, S., KELLY, S., CANNAVAN, A. AND SINGH, D.K. (2021). Recent trends in the use of FTIR spectroscopy integrated with chemometrics for the detection of edible oil adulteration. Vibrational Spectroscopy 113, 103222.

ARIF, M., CHILVERS, G., DAY, S., NAVEED, S.A., WOOLFE, M., YE RODINOVA, O., POMERANTSEV, A.L., KRACHT, O., BRODIE, C., MIHAILOVA, A., ABRAHIM, A., CANNAVAN, A., AND KELLY, S.D. (2021). Differentiating Pakistani long-grain rice grown inside and outside the accepted Basmati Himalayan geographical region using a 'one-class' multi element chemometric model. Food Control, DOI: 10.1016/j.foodcont.2020.107827.

MCGRATH, T.F., HAUGHEY, S.A., ISLAM, M. AND ELLIOTT, C.T. (2021). The Potential of Handheld Near Infrared Spectroscopy to detect food adulteration: Results of a global, multi-instrument inter-laboratory study. Food Chemistry, doi.org/10.1016/j.foodchem.2020.128718.

JAMWAL, R., AMIT, KUMARI, S., BALAN, B., KELLY, S., CANNAVAN, A. AND SINGH, D.K. (2021). Rapid and non-destructive approach for the detection of fried mustard oil adulteration in pure mustard oil via ATR-FTIR spectroscopy-chemometrics. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 244, 118822.

MAESTRONI, B, BESIL, N., REZENDE, S., LIANG, Y., GEREZ, N., KARUNARATHNA, N., ISLAM, M., HEINZEN, H., CANNAVAN, A. AND CESIO, M.V. (2021). Method optimization and validation for multi-class residue analysis in turmeric. Food Control, 121, 107579.

EXTRA-BUDGETARY SUPPORT

PEACEFUL USES INITIATIVE (PUI). Enhancing Capacity in Member States for Rapid Response to Food Safety Incidents and Emergencies, funded by Japan.

PEACEFUL USES INITIATIVE (PUI). Food Authenticity Laboratory Network (FALNET), funded by the U.S.A.

THE INSECT AND PEST CONTROL LABRATORY

EXECUTIVE SUMMARY

In the Genetics and Molecular Biology (GMB) group of the Insect Pest Control Laboratory (IPCL), a new "generic" or "neoclassical" protocol for the development of genetic sexing strains (GSS) was introduced. As a first step, genes responsible for certain phenotypes that could be used as markers are identified and characterized. This is followed by identification of the orthologous genes in different insect species of sterile insect technique (SIT) importance. Next, using modern molecular-based techniques, called "molecular scissors", mutations are introduced to induce the desired phenotypic changes. Finally, a rescue allele (wild type) is transferred in a well-characterized chromosomal region located only in male insects. This method is more effective than the 'classical approach", as in the classical method the detection and isolation of suitable mutations and selectable markers depends on an entirely random process, as well as the induction and selection of a good translocation line.

In addition, the causal gene for the red eye phenotype was identified and mapped, and the developed red eye GSS of *Aedes aegypti* was introgressed into different genomic backgrounds.

Staff of the Livestock Pest (LP) group has started work with the newly acquired self-contained gamma irradiator, FOSS Model 812. Dose variability was identified in the irradiation cannister used for tsetse and assessed as induced sterility in untreated females after mating with males emerging from pupae in different positions in the cannister. Pupae in the bottom of the cannister received the lowest radiation dose, those at the top the highest. The FOSS Model 812 is currently being used for the sterilization of *Glossina palpalis gambiensis* pupae that are biweekly shipped to the tsetse eradication programme in Senegal. The target minimum dose was adjusted to 108 Gy, to reduce overexposure and a 10% increase in operational flies were reported from the programme in Senegal.

The protocol for the sex sorting of tsetse pupae that was available for *G. p. gambiensis* was expanded and modified for *Glossina pallidipes* and *Glossina fuscipes fuscipes*.

Presence of a new bacteria, *Spiroplasma,* reduced productivity of *G. f. fuscipes* colony females and prolonged their reproduction cycle. However, there was no impact of the presence of *Spiroplasma* on the densities of *Trypanosoma* and *Wigglesworthia* in wild *Glossina tachinoides* flies.

In an extensive study using 6000 tsetse flies from 10 different species and collected from 15 different countries in West and East Africa, the relation between *Sodalis* and *Trypanosoma* was assessed. The data indicate that the interaction between *Sodalis* and *Trypanosoma* infection is complex and depends on tsetse species and location.

In the Human Disease Vectors (HDV) group, a comparison was made between 2 larval tray-rack systems, i.e. the Wolbaki and the reference FAO/IAEA rack. Production rates of *Ae. aegypti* and female size were similar for the two systems, but female contamination and male flight ability was slightly better with the reference rack.

Initial results indicated that predation tests with geckos and mantis species have great potential as an addition to routine quality control tests of male mosquitoes.

Gafchromic™ films have been a standard component of irradiation work, and newer versions of the high dose and medium dose films were evaluated. They showed that the response to these films is no longer sensitive to temperature and the films have improved in consistency of the absorption

spectrum at various doses. These improvements will simplify the use and processing of the films for dose measurement.

Staff of the IPCL recently evaluated an "off-the-shelf" X-ray blood irradiator (Raycell MK2) for its suitability for sterilizing insects in the frame of the SIT. The findings show a very high dose uniformity of 1.14 and thus a small range of variation of dose distribution within the irradiation canister, suggesting that this technology can be adopted for small to medium-scale SIT programmes.

In the Plant Pest (PP) group, work in support of the USDA/IAEA agreement on phytosanitary treatments continued targeting quarantine fruit fly species. During a phytosanitary cold treatment against *Zeugodacus tau,* a total of 36,512 third instar larvae infesting navel oranges were exposed to ≤ 1.7 °C for 22 days, yielding four survivors that failed to emerge as adults

Two new protocols were developed to assess competitiveness of mass-reared sterile male *Drosophila suzukii* under ecologically realistic conditions. One method was based on mating observation and a second relies on the individualization of fertile females and the evaluation of their fertility. The second method was identified as a credible alternative to the first.

A self-contained ^{60}Co irradiator (FOSS 812) and the Raycell MK2 X-ray blood irradiator were used in *Drosophila suzukii* sterilization studies. The radiation source did not affect the level of sterility after irradiation of the pupae with 170 and 220 Gy (doses proposed for *D. suzukii* suppression or eradication) under low oxygen conditions. Similarly, the radiation source did not affect fly emergence and sex ratio, flight ability, survival under stress, and mating competitiveness.

A range of gamma radiation doses were administered to pupae from a bisexual strain and a new black pupae GSS of *Anastrepha fraterculus* under hypoxia. Complete sterilization was achieved with a dose of 80 Gy and the quality of the sterile insects in terms of adult emergence was similar between strains, but slightly lower in comparison with an untreated control group.

In terms of capacity building, in 2021 the IPCL hosted two cost-free experts and 15 consultants of which 9 were PhD students, 13 interns, two fellows and one scientific visitor. The fellows were funded by the IAEA'S Department of Technical Cooperation.

The GMB and PP groups carried out 68 fruit fly shipments to 23 institutions in 15 countries and six shipments of preserved fruit flies to six countries. The LP group carried out 116 tsetse shipments of 81,458 pupae to seven institutions in five countries. The HDV group carried out 22 mosquito shipments to eight institutions in seven countries.

In 2021, the IPCL received 106 official visitors from 19 countries.

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Adun, Henry	Laboratory Technician (LP)
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MAJOR ACHIEVEMENTS AND DEVELOPMENT

Genetics and Molecular Biology

A generic (neoclassical) approach for the development of genetic sexing strains

The sterile insect technique (SIT) is based on the release of sterile male insects into the wild where the males will mate with the wild females, leading to the suppression or local eradication of the target population. If sterile female insects are released, the efficacy and cost-effectiveness of the technique is reduced as the sterilized released insects tend to mate with each other. In addition, released females can still cause damage to fruit and vegetables in the case of insect plant pests, or transmit microbial pathogens such as with insect disease vectors. To accomplish male-only releases, genetic sexing strains (GSSs) have been developed, thus enhancing the overall effectiveness of SIT applications in the frame of area-wide insect pest management (AW-IPM) programmes.

For the development of a GSS strain, two prerequisites should be fulfilled. First, a selectable marker is identified that induces morphological (e.g. colour of the pupae) or conditional (e.g. lethality at elevated temperatures) changes to the insects. Second, a wild type (rescue) allele of the selectable marker is linked to a male-specific region. The **classical approach** to produce GSSs is achieved in three steps. The first step includes the identification of a marker. The marker can be found naturally in the population, for example through visual screening, or it can be induced by chemical/irradiation-based treatments to wild-type strains. The second step involves the linkage of the wild type (WT) allele of the marker to the male sex by irradiation-induced translocations. After applying an appropriate scheme of crosses and backcrosses, individuals with the dominant wild-type allele pseudo-linked to the Y-chromosome (or to the chromosomal region which carries the male-determining locus) are identified and used for the development of a strain that produces phenotypically wild-type males and mutant females (Fig. 1).

Although extremely successful GSSs have been developed using the classical approach, such as the *Ceratitis capitata* VIENNA strains, it is still very challenging, labour intensive and time consuming to develop novel GSSs following this method. The reason is that the detection and isolation of suitable mutations and selectable markers depends on an entirely random process as well as the induction and selection of a good translocation line. In addition, the translocation lines are semi-sterile as half of the male gametes are genetically unbalanced. To overcome these challenges, a new, more effective process for the development of GSSs is needed.

In the frame of the CRP D44003 on "Generic approach for the development of genetic sexing strains for sterile insect technique (SIT) applications", a new approach called "**generic**" or "**neoclassical**" is introduced. First, genes responsible for certain phenotypes (e.g. colour of pupae or temperature sensitive lethality) that could be used as markers are identified and characterized, including the genetic basis of associated mutations (e.g. the white colour of a tephritid pupae instead of the wild-type brown colour). The next step is to identify the orthologous genes in different insect species of SIT importance. In this framework, the genetic basis of the *white pupae (wp)* marker that had already been used for the GSSs for *Ceratitis capitata*, *Bactrocera dorsalis* and *Zeugodacus curcubitae* was recently discovered. Next, using the modern molecular-based techniques of "molecular scissors", mutations are introduced to induce the desired phenotypic changes. Additionally, using the same tools, a rescue allele (wild type) is transferred in a well-characterized chromosomal region located only in male insects (Fig. 1). In this way, the genetic stability of the strains is not interrupted and new GSSs are developed in a more targeted, straightforward, and faster way.

After the detection of the genetic basis of the *white pupae (wp)* marker, research efforts are focused on two directions: the discovery of the molecular basis of genes responsible for other key phenotypic traits and the identification of male-specific region(s) to link the wild-type allele of the marker gene. Additional candidate markers include: (a) the *temperature sensitive lethal (tsl)* gene, used in addition with the *wp* gene, as a selectable marker for VIENNA 7 and VIENNA 8 GSSs of *C. capitata*, (b) the *black pupae (bp)* gene, which is currently the only selectable marker for the *Anastrepha ludens* and *Anastrepha fraterculus* GSS and (c) the *red eye (re)* gene, which has been used in the respective GSS of *Aedes aegypti*. Advances on these directions can set the ground for the development of more efficient and enhanced-quality GSS strains.
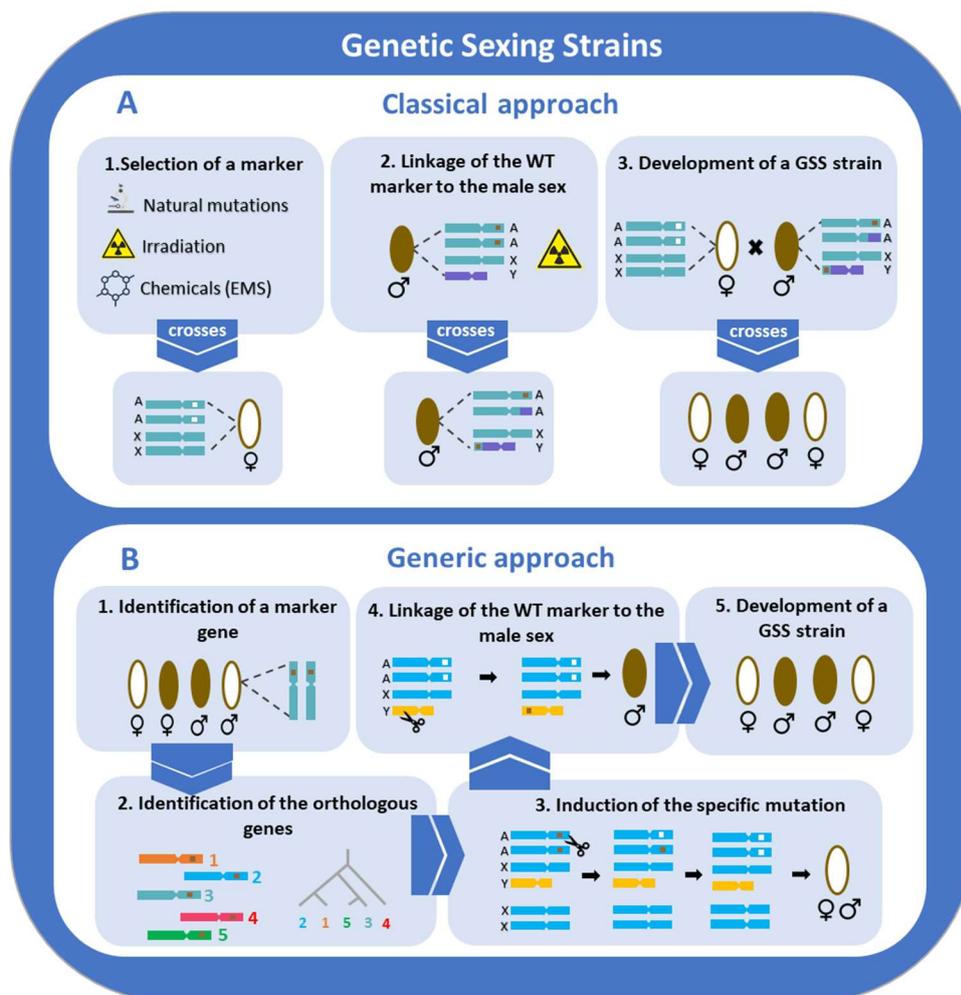
*FIG. 1. An overall presentation of the experimental procedure for the development of GSSs strains using: A. The **classical approach** that includes the (1) the selection of a marker, (2) the linkage of the WT marker to the male sex and (3) the development of a GSS. Each step includes a specific scheme of crosses and backcrosses. B. The **generic (neoclassical) approach** that includes: (1) the identification of the genetic basis of a marker, (2) the identification of orthologous genes in other insect species, (3) the induction of a mutation through modern biotechnological tools and the establishment of a homozygous strain with the desired phenotype, (4) the linkage of the rescue allele (wild type) on the Y chromosome (or to the chromosomal region which*

carries the male determining locus) through modern biotechnological tools, and (5) the establishment of the new GSS strain via the generic (neoclassical) approach

Discovery of Aedes aegypti *red eye gene*

The *red eye (re)* gene of *Aedes aegypti* has been characterized as a recessive, sex-linked (mapped to chromosome I) gene that presents full penetrance and expressivity. The gene is closely linked to the M locus and the genetic distance has been estimated to 2-3 cM (Fig. 2). Despite this prior knowledge, the causal gene for the red eye phenotype in *Ae. aegypti* was not known until recently.

Aedes aegypti has an overall lower per megabase recombination rate while its chromosome centromeres are also surrounded by low recombination regions. These two combined can make the process of mapping genes responsible for particular traits, such as the eye colour, quite challenging. In the frame of the coordinated research project (CRP) on "Generic approach for the development of genetic sexing strains for sterile insect technique (SIT) applications" and in collaboration with Prof. Jake Tu's research group (member of the CRP), a marker-assisted-mapping (MAM) strategy was developed. The new strategy allows the screening and genotyping of the rare but informative recombinants, thus enhancing the resolution and signal-to-noise ratio and overcoming the low recombination rate barriers (Chen et al., bioRxiv 2021, doi: https://doi.org/10.1101/2021.04.29.442065).
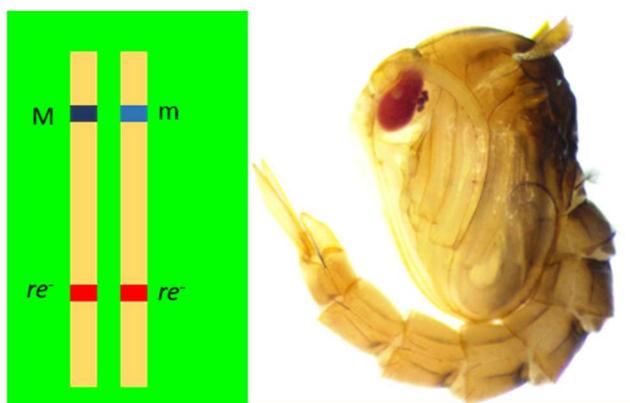
FIG. 2. Genotype and phenotype of an Aedes aegypti *red eye male at the pupal stage.*

Using *Ae. aegypti* red-eye genetic sexing strains introgressed into different genomic backgrounds developed at the IPCL, the *red* genetic locus was mapped to the region between 271 Mbp and 278 Mbp of chromosome I and the possible *red eye* gene candidates included in this area were evaluated. Following functional genetic approaches, *cardinal* was identified as the causal gene of the red eye colour in *Ae. aegypti*, a finding that was also supported by a similar study on the diamondback moth *Plutella xylostella*.

Cardinal encodes a peroxidase which catalyses the formation of the eye pigment xanthommatin from 3-hydroxykynurenine in the ommochrome synthesis pathway. The identification of the *cardinal* gene is expected to be of service in the efforts towards the development of GSS for several mosquito species, including *Ae. albopictus* and *Anopheles* species, using the generic (neoclassical) approach described above.

Introgression of Aedes aegypti *red-eye genetic sexing strains into different genomic backgrounds*

Aedes aegypti is widely recognized as a major vector of arthropod-borne viruses (arboviruses) that trigger severe health, societal and economic problems in the areas in which it is thriving. The SIT as a component of AW-IPM programmes has been suggested as a sustainable population suppression strategy. However, the implementation of an SIT programme for mosquitoes first and foremost requires an efficient and robust method for sex separation, since female mosquitoes are the ones that bite, blood feed, and potentially transmit the pathogens.
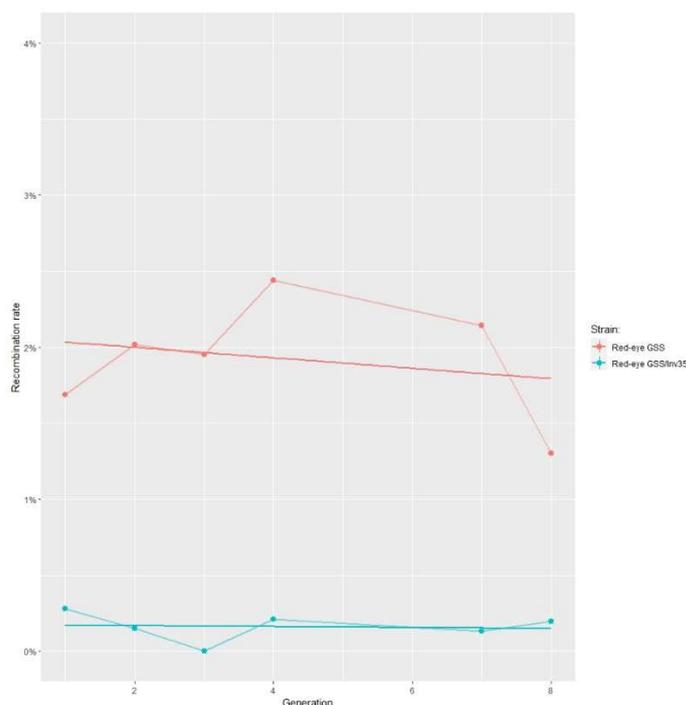
FIG. 3. Recombination rate data collected during several generations of the Red-eye GSS and Red-eye GSS/Inv35 Aedes aegypti *strains. The straight line represents the fitted linear model.*

The *Ae. aegypti* Red-eye GSS that has been developed by the IPCL is based on a red-eye morphological marker. The marker allows the separation of black-eye males from red-eye females, thus enabling the implementation of an SIT programme. The *Ae. aegypti* Red-eye GSS has been constructed through classical genetics and its quality control profile demonstrated its remarkable performance and genetic stability under laboratory conditions. The genetic stability of the strain was further improved by the induction of an irradiation-based chromosomal inversion (Inv35), covering the red-eye genomic region,drastically reducing the probability of female contaminants in the male-release batches. However, a GSS could still face performance reduction when released in the field due to the different genomic background with the local strain. As a rule of thumb, it has been suggested that developing mosquito GSS that will be integrated into the local genomic background of the release area will not only maximize the successful matings between released males and wild females, but will also address potential biosafety and biosecurity concerns. Therefore, any future operational SIT programme that will employ the Red-eye GSS (either with or without the inversion) should ideally be carrying the local genomic background of the targeted population.

Different *Ae. aegypti* wild populations were used to introgress the *Ae. aegypti* red-eye mutation and the inversion Inv35 lines through a series of genetic crosses. As a result, Red-eye GSS and Red-eye

GSS/Inv35 strains with local genomic backgrounds were developed from six different geographic locations and their genetic stability was assessed for several generations. The results indicated that the genetic sexing properties of the strains were not affected, while in all Inv35 GSS strains the recombination events were drastically suppressed (Fig. 3). Maintaining the local background also minimizes any potential effects on mating behaviour and enhances the odds for increased male mating competitiveness. In addition, release of mosquitoes that carry the same local genomic background as their wild counterparts addresses any potential biosafety concerns and significantly enhances the public acceptance towards AW-IPM programmes with an SIT component.

Livestock Pests

Tsetse colonies

The IPCL maintain colonies of tsetse to meet the needs and requirements of IAEA and FAO Member States. At present, colonies of seven tsetse species, i.e., *Glossina pallidipes, Glossina morsitans morsitans, Glossina morsitans submorsitans, Glossina morsitans centralis, Glossina brevipalpis, Glossina fuscipes fuscipes* and *Glossina palpalis gambiensis* and 8 strains are maintained at the IPCL.

The *G. p. gambiensis* colony is the largest, and has been maintained to conduct research in support of a project to create a zone free of tsetse in the Niayes area located north of Dakar, Senegal. The colony is currently maintained at a level of 30 000 producing females to provide research material and to supply operational sterile males currently deployed in the tsetse eradication campaign in Senegal. The *G. f. fuscipes* colony is the second-largest colony and provides material to conduct research for the preparation of the SIT for the control of the human African trypanosomosis vector in Chad.

The FOSS Model 812 Co-60 self-contained irradiator used in tsetse SIT

The FOSS Model 812 ^{60}Co self-contained irradiator is a new addition to the irradiator resources of the IPCL. The system has a large capacity and can deliver variable dose rates. The model has three separate Cobalt 60 sources that can be used individually or in combination. The samples can also be positioned on three turntables of various diameters with up to eight times attenuation to provide dose rates from milliSv to kiloSv/per minute.

As the success of the SIT depends on the release of competitive, sterile males into the natural habitat of the species targeted for control, it was necessary to assess the sterility and quality of the males derived from the pupae irradiated with the FOSS Model 812 irradiator.

The FOSS Model 812 characterization and dose mapping were done by Mr Yeudiel Gomez Simuta, a consultant from the Moscafrut Programme, Mexico. Following his dose-tracking recondition, tsetse pupae were irradiated with a target minimum dose of 118 Gy in air at room temperature inside the pupal shipment box. The box was placed in the vertical position. The pupae were exposed with all three ^{60}Co sources in the turntable three position which led to a dose rate of 78.63 Gy/min. To monitor the radiation dose, HD-V2 Gafchromic film (mean uncertainty 4.36% at 95% CI) was used. HD-V2 films were placed in small white envelopes at three different levels at the bottom, middle and top of the pupal shipment box as can be seen in the figure below. An optical density meter (DoseReader 4, RadGen, Budapest, Hungary) was used to read the HD-V2 films, 24 h after exposure. A pupal sample was collected from the bottom, middle and top of the pupal shipment box for emergence and sterility assessment.

The HD-V2 film readings indicated that the lowest dose of 103 Gy was observed at the bottom of the shipment box and the highest dose of 142 Gy at the top. The pupae at the bottom of the box received

an average dose of 120±6.0 Gy, in the middle 131±8.3 Gy and at the top 130±6.9 Gy, all above the minimum target dose of 118 Gy.

The emergence rate of the pupae sampled from the bottom, middle and top of the pupal shipment box was similar and averaged between 75±7.9% and 77±6.1%. The males that emerged from the selected pupae were mated 1:1 with virgin females. Reproduction of the females was monitored for 60 days and the sterility induced in the females determined. The lowest induced sterility (92.8%) was recorded in the males selected from the bottom of the box. On average the induced sterility of the males selected from the bottom of the box was the lowest (96.7±1.9%), followed by males from the middle (97.7±1.4%), and the highest was for males selected from the top of the box (98.9±1.2). Due to the unavoidable dose variability within the canister, highlighted again with the data above, many of the pupae received a higher radiation dose than intended and this will decrease male quality and competitiveness.

The FOSS Model 812 is currently being used in the sterilization of *G. p. gambiensis* pupae that are biweekly shipped to the tsetse eradication programme in Senegal. The target minimum dose was adjusted to 108 Gy, to reduce overexposure and a 10% increase in operational flies was reported from the programme in Senegal.

FIG. 4. Ms Arooj Nawaz preparing the male Glossina palpalis gambiensis *pupae to be sterilized with the FOSS Model 812 irradiator.*

A protocol for sex sorting of tsetse pupae with the Near Infrared Pupal Sex Sorter (NIRPSS)

The newly developed Near InfraRed Pupal Sex Sorter (NIRPSS) allows sex separation of tsetse pupae and the process is currently being used for shipping male *Glossina palpalis gambiensis* pupae to the eradication programme in Senegal. The pupae can now be sorted five days before emergence of the adults and can be shipped long distance without using low-temperature conditions to prevent emergence. This has resulted in a 20% increase in male fly quality in the Senegal tsetse eradication programme. The sorting protocol used for *G. p. gambiensis* requires pupae to be incubated at a constant temperature of 25°C and relative humidity of 80%. The pupae can then be sorted with an accuracy of 90% when the pupae are 24 days old, or about 6 days before emergence.

The sorting protocol was evaluated and extended to include *Glossina pallidipes*, a target vector in Ethiopia, and *Glossina fuscipes fuscipes*, a target vector in Chad. The pupae that were sorted were all deposited within a 10-hour period and incubated at a constant temperature and relative humidity of 25°C and 80%, respectively. The pupae were sorted daily with the NIRPSS when they were between 22-26 days old. A melanized to non-melanized ratio of 70:30 was needed to achieve a 90% sex accuracy, and this was achieved on day 24 post-larviposition for *G. pallidipes* pupae and on day 26

post-larviposition for *G. f. fuscipes* pupae. In a subsequent evaluation, the effect of the sorting with the NIRPSS on the quality of the males was also evaluated and in both species, no significant wing damage could be found and there was no significant negative affect on the males' ability to fly.

The NIRPSS sorting protocol for three tsetse species is now available and will soon be extended to include *Glossina brevipalpis*, a target vector in southern Africa.

FIG. 5. Ms Olga Soukia collecting the "fliers" of the tsetse flight propensity quality control test, where the effect of NIRPSS on the quality of the males was assessed.

The impact of Spiroplasma *infection on the performance of a* Glossina fuscipes fuscipes *colony*

Tsetse flies are known to harbour a unique bacterial community, mainly consisting of the obligate *Wigglesworthia glossinidia*, the commensal *Sodalis glossinidius*, and the widespread symbiont *Wolbachia pipientis*. Recently a fourth bacteria, "*Spiroplasma*", was found to infect tsetse flies from the *palpalis* group. The prevalence of *Spiroplasma* infection and its impact on the performance of *Glossina fuscipes fuscipes* was analysed by Mr Kiswenda-Sida Mikhailou Dera, a PhD student from Burkina Faso in collaboration with Prof Serap Aksoy and Dr Brian Weiss from Yale University, USA. The impact of *Spiroplasma* on female productivity and mating ability was determined and the results indicate that *Spiroplasma*-infected females produced fewer pupae in comparison with uninfected females.
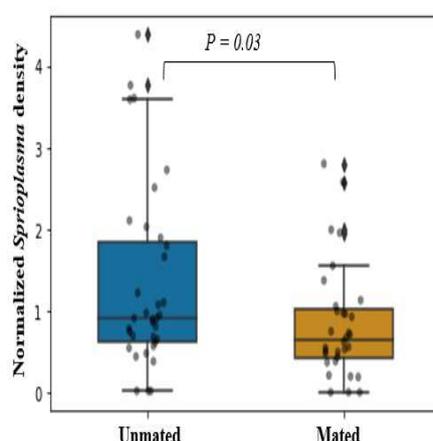
FIG. 6. Normalized Spiroplasma *density in mated and unmated* Glossina fuscipes fuscipes *flies.*

The results also indicate that the pregnancy cycle in *Spiroplasma*-infected females was longer than that of uninfected females. Moreover, unmated adults showed a higher density of *Spiroplasma* than mated adults which might indicate that *Spiroplasma* reduced the mating ability of the adults (Fig. 6). Analysing the insemination rate and the spermatheca fill indicated a lower number of fully-filled

spermathecae with the seminal fluid in *Spiroplasma*-infected females, as compared with uninfected females. To further explore the impact of *Spiroplasma* on the performance of the *G. f. fuscipes* colony, colonies with low and high levels of *Spiroplasma* infection (Sp- and Sp+) were established and screened by non-destructive PCR method. The performance of these colonies is currently being assessed.

Prevalence of Spiroplasma *infection in wild* Glossina tachinoides *populations*

The prevalence of *Spiroplasma* infection was assessed in wild *Glossina tachinoides* populations using primers to amplify the 16S rRNA, and the results indicate an overall high prevalence (Fig. 7). However, using other primers to genotype *Spiroplasma* strains showed a lower prevalence which might indicate that different strains are circulating in the tested populations. The genotyping and assessing of the number of *Spiroplasma* strains in the tested population is currently in progress. This work was done by Mr Moustapha Dieng, a consultant from Senegal.
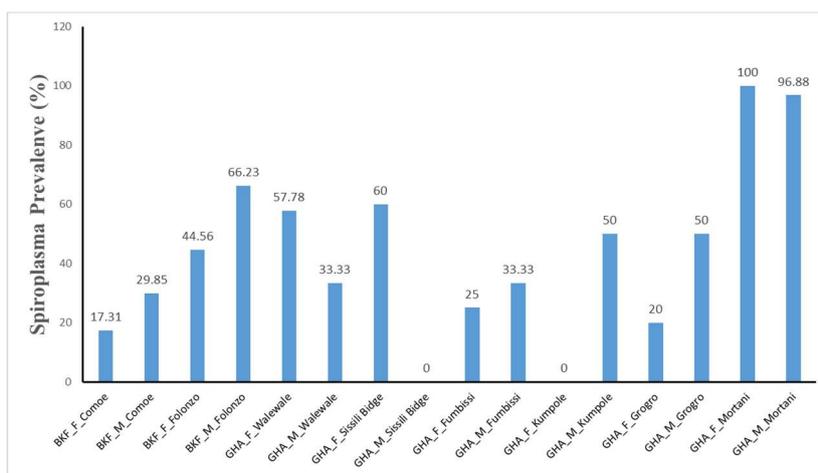
FIG. 7. Prevalence of Spiroplasma *infection in wild* Glossina tachinoides *tsetse flies in Ghana (GHA) and Burkina Faso (BKF).*

The impact of Spiroplasma *infection on* Wigglesworthia *and* Trypanosoma *density in wild populations of* Glossina tachinoides

The interaction between *Spiroplasma* and the tsetse fly symbionts *Wigglesworthia, Sodalis* and *Wolbachia,* the salivary gland hypertrophy virus (SGHV) and *Trypanosoma* remains to be elucidated.
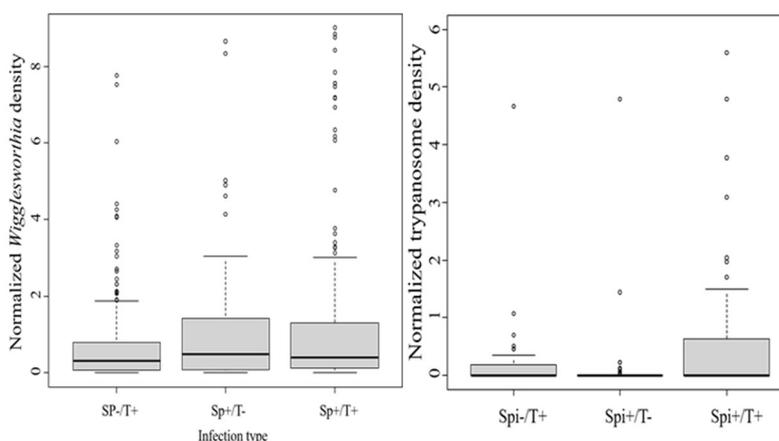
FIG. 8. Interaction between Spiroplasma *infection on* Trypanosoma *(right) and* Wigglesworthia *(left) density in wild* Glossina tachinoides *in West Africa.* T: Trypanosoma*;* Sp: Spiroplasma*.*

To explore these interactions, we screened wild tsetse population of *G. tachinoides* collected from Burkina Faso and Ghana for the presence of these organisms. The results indicated the absence of *Sodalis*, *Wolbachia* and SGHV in these flies. However, some flies were infected with *Spiroplasma* or

Trypanosoma or both. We analysed the impact of *Spiroplasma* infection on *Trypanosoma* and *Wigglesworthia* density in wild *G. tachinoides* using relative qPCR. The results indicated no significant impact of *Spiroplasma* infection on *Trypanosoma* and *Wigglesworthia* densities (Fig. 8).

Isolation and purification of iflavirus and negevirus particles in Glossina morsitans morsitans

Some tsetse fly species such as *Glossina morsitans morsitans* and *Glossina morsitans centralis* harbour RNA viruses (iflavirus and negevirus) in addition to the four symbiont bacteria. The impact of these viruses on the productivity of tsetse colonies is under investigation. To better identify these viruses, attempts were made to isolate and purify the virus particles from *Glossina morsitans morsitans* using the sucrose gradient ultra-centrifugation method. Thereafter, the purified viruses were observed under transmission electron microscope (Fig. 9). The observed viral particles were morphologically similar to the iflavirus detected in honey bees. PCR results confirmed the presence of iflavirus and negevirus and therefore further steps are needed to enable the separation of the two viruses. This work was conducted by Ms Hannah Huditz, a PhD student from Austria in collaboration with Prof. Monique van Oers, Wageningen University.
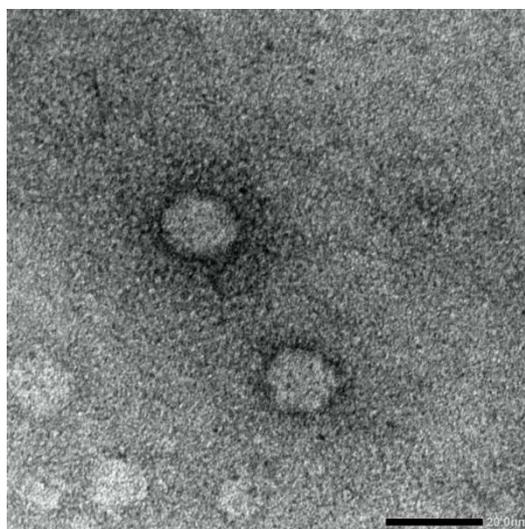
FIG. 9. *Purified viral particles isolated from* Glossina morsitans morsitans *infected with iflavirus and negevirus observed under electron microscope (scale bar 20nm).*

The interaction between Sodalis *and* Trypanosoma *infections in wild tsetse population at continental level.*

Sodalis infection was reported in many tsetse species and its interaction with *Trypanosoma* infection in tsetse fly has been ambiguous and not clear. To elucidate these interactions, more than 6000 flies belonging to 10 different tsetse species and subspecies were collected from 15 countries in sub-Saharan countries and analyzed for *Sodalis* and *Trypanosoma* prevalence (Fig. 10). The data clearly indicate that the interaction between *Sodalis* and *Trypanosoma* infection is complex and depends on tsetse species and location. The analysis did not allow drawing a general conclusion. The data was submitted and accepted for publication in Scientific Report Journal.
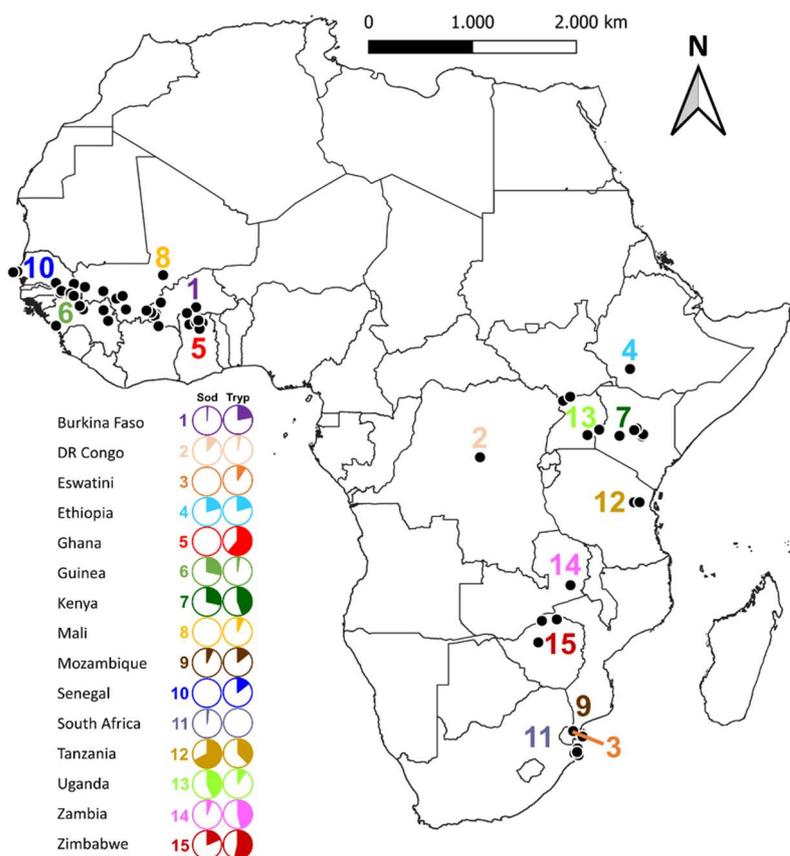
FIG. 10. The geographical locations of tsetse samples in Africa. Circles indicate the total prevalence of Sodalis and Trypanosoma per country. Black dots indicate sample collection site(s) per country.

Human Disease Vectors

The work of the Human Disease Vectors group of the IPCL has focused on improving the various steps of the SIT package against mosquitoes and transferring knowledge, protocols and training materials to support capacity building in Member States towards pilot suppression trials in selected field sites.

The efficiency of the Wolbaki™ mass-rearing tray-rack system as compared with the reference FAO/IAEA aluminium mass-rearing rack

The operational success of the SIT as a component of AW-IPM depends on being able to continuously produce and release good-quality sterile males in large enough numbers to achieve appropriate sterile-to-wild male ratios. Since 2004, following the request from IAEA Member States, the Joint FAO/IAEA IPCL has been developing equipment and protocols for the application of the SIT against disease-transmitting mosquitoes. Significant progress has been made in the development of such equipment and protocols and many SIT pilot trials are now ongoing in various countries around the world. However, for the economic viability and sustainability of these SIT programmes, efforts to improve the existing technologies should be continuously maintained to reduce the cost of sterile male' production and increase their efficiency.

The Chinese company Wolbaki Biotech has developed a new larval mass-rearing rack prototype based on the FAO/IAEA reference rack model (see figure below) to mass-rear *Aedes albopictus*. This Wolbaki model consists of a mechanized stainless-steel rack able to hold up to 100 rearing trays (smaller than

the FAO/IAEA large trays). Trays are stacked in two blocks of 50 in the whole rack, covering a surface area of 1.1 m^2 in comparison with the FAO/IAEA mass-rearing rack's 0.71 m^2. The IPCL was provided with one Wolbaki rack to assess its efficiency for rearing *Aedes aegypti*.

Experiments were designed to (1) estimate and compare the pupae production (male and female) per rearing tray, rack and per surface unit and (2), estimate and compare the female contamination rate, body size and male flight ability.

Preliminary results show that the production rates of adult males and females that emerged from pupae maintained in the FAO/IAEA and Wolbaki rack systems, as well as female/male body size, were similar. However, the percentages for male/female contamination and male flight ability differed slightly between the two systems in favour of the FAO/IAEA unit. Moreover, in terms of production per surface unit and cost, the FAO/IAEA appeared more cost effective than the Wolbaki rack. Notwithstanding the above, considering the variability between rearing protocols, mosquito strains and our present results, the Wolbaki rack (Model WBK-P0003-V2) can still be considered as a useful piece of equipment that can be used efficiently for mass-rearing mosquitoes without impacting the production and their quality within operational SIT programmes.

FAO-IAEA Aluminium rack   Wolbaki tray-rack system

FIG. 11. The FAO/IAEA (left) and Wolbaki (right) rearing rack systems.

Validation of the IPCL adult mass-rearing cage for Aedes albopictus *and* Anopheles arabiensis

Most mosquito-rearing facilities dedicated to SIT programmes are currently occupied with developing improved tools and methods to enhance the capacity for mass rearing the local mosquito strains in sufficient numbers and are often limited by the inadequate size of readily available mosquito cages, which are commonly 30 × 30 × 30 cm or 60 × 60 × 60 cm for small-scale releases. However, the production capacity of mass-rearing insectaries for mosquitoes has been increasing rapidly in recent years. Mass-rearing and release facilities for *Aedes* mosquitoes are currently being built in several countries and technical and economic decision-making guidelines associated with facility design, cost, construction, equipment, and operation have been developed. Successful implementation of SIT against *Aedes albopictus* and *Anopheles arabiensis* relies on a continuous supply of sterile males. To meet this requirement, optimization of the mass-rearing techniques is needed. The IPCL has recently developed a plexiglass mass-rearing cage (MRC) for *Aedes aegypti*. The MRC was tested for other species, namely *An. arabiensis* and *Ae. albopictus*. Findings suggest that the new MRC prototype is

efficient in terms of egg production and can be used for mass rearing in SIT programmes targeting *Ae. albopictus* as well as *An. arabiensis*. Although the new MRC has shown several advantages, further improvements are ongoing to increase efficiency and stacking ability of several cages in a mass-rearing facility.

Adult mosquito predation and potential impact on the sterile insect technique

A good understanding of the interactions between adult mosquito predators and laboratory-produced mosquitoes is important to better define the requirements of the genetic control strategies included in the SIT. The predation propensity of four mantis species (*Phyllocrania paradoxa*, *Hymenopus coronatus*, *Blepharopsis mendica*, *Deroplatys desiccate*) and two gecko species (*Phelsuma standingi*, *P. laticauda*) on adult *Ae. aegypti*, *Ae. albopictus* and *An. arabiensis* mosquitoes were investigated under laboratory settings. Effects of different mosquito characteristics and treatments, including species, sex, chilling, marking and irradiation on predation rates were assessed. All tested predators effectively preyed on all mosquito species whatever the treatments. However, the predation propensity varied over days for the same individuals and between predator individuals. Mosquito characteristics including species, sex, chilling and marking did not affect their vulnerability to mantis predation. However, high doses of irradiation can make mosquitoes more vulnerable to mantis predation. Therefore, a trade-off must be found between sterility and quality to optimize SIT programmes. Overall, the mantis species *B. mandica* showed more predacious capacity than the others. The gecko *P. standingi* also preyed upon all mosquito treatments with a relatively high predation rate. It was found to prefer eating *An. arabiensis* than *Ae. aegypti* and *Ae. albopictus* but caught the other paired mosquito-treatments at a similar rate. *Phelsuma laticauda* predated similarly upon *Aedes* species but showed a trend to prefer *Ae. aegypti* to *An. arabiensis*. Our study pointed out the potential impact of predators on the survival of laboratory-produced males to be released in SIT programmes. Standardized predation trials may become useful additional quality control tools of irradiated mosquitoes.

FIG. 12. Blepharopsis mendica *eating a female* Aedes *mosquito (Photo: T. Wallner).*

Impact of chilling and anoxia on irradiation of adult mosquitoes

Following the series of experiments to identify physical and biological factors that affect dose-response in mosquito pupae, an assessment of the impact of external factors on adult mosquito dose response has been initiated. Preliminary results on *Aedes albopictus* show that immobilizing methods such as chilling and anaesthetizing agents affect direct radiation effects and can also alter downstream male-quality parameters. Chilling induces damage in the insects, but partial or full recovery is possible if chilling duration and temperature are carefully controlled. Irradiation in nitrogen has high radioprotective effects in adults and higher irradiation doses are needed to achieve the desired sterility level. Longevity of males can be improved by irradiating in anoxia, however the exposure to nitrogen itself comes with negative impacts on flight ability. Recovery of flight ability did not occur within 2 days. However, longer recovery phases were not tested. Irradiation in anoxia improves male

mating competitiveness as has been reported in other insects but has yet to be fully evaluated in mosquitoes.

Use of Gafchromic™ films for dosimetry

Dosimetry is an essential component of any irradiation study. For any chosen dosimetry system, such as the use of radiochromic film, accuracy and reliability need to be warranted. For irradiation studies at the IPCL, Gafchromic™ films have been a standard component of irradiation work. Previously, the characteristics of the film included a sensitivity to temperature during radiation exposures, and a temperature correction was applied when calculating the absorbed dose. Closer investigation of the newer versions of the high dose (HD) and medium dose (MD) films have recently shown that the response to these films is no longer sensitive to temperature and have improved in consistency of the absorption spectrum at various doses. These improvements will simplify the use and processing of the films for dose measurement.

Assessment of an X-ray blood irradiator and its application potential for the SIT

Self-contained gamma irradiators have been widely used for research as well as for the production of sterile insects in many SIT facilities around the world. More recently, the use of X-ray generators has been investigated as an alternative due to the reduced purchase costs, as well as simplified logistic, regulatory and safety requirements. For both gamma-ray and X-ray irradiation, it is important to assess the homogeneity of the absorbed dose in the samples, and to measure the maximum and minimum dose in an irradiation sample. This can be achieved by dose mapping in a reference process load. The IPCL has recently evaluated an "off-the-shelf" X-ray blood irradiator (Raycell MK2, Best Theratronics) for its suitability for sterilizing insects in the frame of the SIT. The findings show a very high dose uniformity of 1.14 and thus a small range of variation of dose distribution within the irradiation canister, suggesting that this technology can be adopted for small to medium-scale SIT programmes.

Plant Pests

Advances in the development and evaluation of nonchemical phytosanitary treatments targeting quarantine fruit fly species

Confirmatory tests evaluating the efficacy of a phytosanitary cold treatment against *Zeugodacus tau* from Fujian, China were completed. A total of 36,512 third instar larvae infesting navel oranges were exposed to ≤ 1.7 °C for 22 days and yielded four survivors, one larva and three pupae. All survivors failed to emerge as adults. A manuscript summarizing the findings has been drafted and will be submitted for publication in 2022.

FIG. 13. Mr. Fabio Luís Galvão da Silva, an intern from Brazil, dissecting fruit infested by Zeugodacus tau *after exposure to phytosanitary cold treatment.*

Research evaluating dose-rate effects on phytosanitary irradiation efficacy for *Ceratitis capitata* was completed. The phytosanitary irradiation treatment of 100 Gy proved to be effective against third instar *C. capitata* larvae infesting mandarins, regardless of dose rate. Only negligible differences in emergence were observed in irradiation treatments of 20 and 30 Gy, suggesting that dose rate may not affect the efficacy of the phytosanitary irradiation dose used for *C. capitata*. Significant dose distribution differences were observed while conducting dose rate experiments using a Foss 812 gamma irradiator. A paper summarizing these findings will be drafted and submitted for publication.

Research conducted in support of the Technical Panel on Phytosanitary Treatments (TPPT)

Supporting results obtained by IPCL and USDA scientists provided critical technical justification for the recommendation from the TPPT to remove the restriction of phytosanitary irradiation application against fruit flies for commodities stored in modified atmosphere. This recommendation was approved in the fifteenth session of the Commission on Phytosanitary Measures (CPM-15). As a result, the disclaimer "This irradiation treatment should not be applied to fruits and vegetables stored in modified atmospheres" was removed from nine PTs of the ISPM 28, thus making phytosanitary irradiation against fruit flies more broadly applicable.

Confirmatory large-scale tests on phytosanitary irradiation for *Drosophila suzukii,* or the spotted wing drosophila (SWD), have been conducted to validate the radiation dose of 80 Gy. The study was designed and performed to include all requirements and suggestions from the TPPT, which were published in its report of the July 2019 meeting. Briefly, naturally infested blueberries containing late-stage pupae were irradiated with 80 Gy of gamma rays and held in the laboratory to assess adult emergence, mating, and oviposition. More than 30 000 late-stage pupae of *D. suzukii* have been treated (Fig. 14). A few emerged adults from the radiation treatment were able to lay eggs but these did not hatch, indicating no development to a F1 generation. Currently, there are neither adopted irradiation treatment schedules nor sufficient phytosanitary irradiation studies to support irradiation treatment schedules for *D. suzukii*. Results generated from this project have the potential to support an irradiation treatment recommendation to the TPPT that, if approved and adopted, could benefit the Member States of the FAO and IAEA.
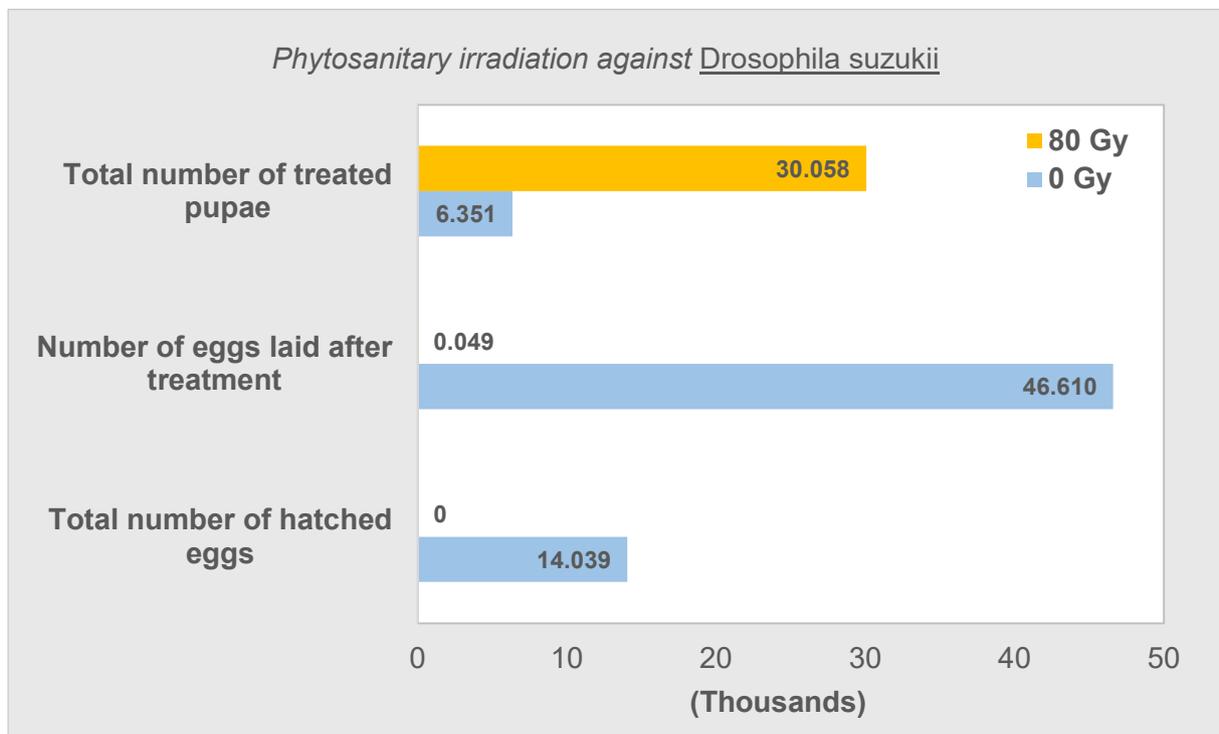
FIG. 14. *Large-scale confirmatory tests with* Drosophila suzukii *late-stage pupae in blueberries to validate the radiation dose of 80 Gy as a phytosanitary irradiation treatment for the species.*

Drosphila suzukii - *New protocols to assess sterile male competitiveness*

Drosophila suzukii is an invasive crop pest that attacks a large range of soft fruits. The SIT is being evaluated as a tool to control populations of *D. suzukii* in confined environments such as greenhouses. Two protocols were developed to assess competitiveness of mass-reared sterile males under ecologically realistic conditions. A method based on mating observation was validated in the spring of 2021. Due to the small size of the insect and its elusive behaviour, a second method that relies on the individualization of fertile females and the evaluation of their fertility was tested in the autumn of 2021 and identified as a credible alternative to the first method.

FIG. 15. Different cages used for the evaluation of the competitiveness of sterile male Drosophila suzukii.

Drosphila suzukii – *Evaluation of X-ray irradiation as an alternative for sterilization*

Drosophila suzukii sterilization has been studied previously using isotopic irradiators. The effects of gamma rays and X-rays on fly sterility and quality were compared. A self-contained ^{60}Co irradiator (FOSS 812; Foss Therapy Services, CA, USA) and an X-ray blood irradiator (Raycell MK2; Best Theratronics, ON, Canada) were used. The radiation source did not affect the level of sterility after irradiation of the pupae with 170 and 220 Gy under low-oxygen conditions; these two doses were proposed previously for SWD suppression or eradication. Similarly, the radiation source did not affect fly emergence and sex ratio, flight ability, survival under stress, and mating competitiveness. These results confirm the possibility of using X-ray irradiators for small SIT projects.
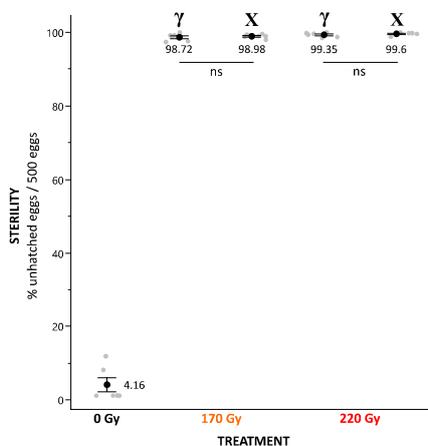
FIG. 16. Percentage of sterility induced in untreated female Drosophila suzukii *after mating with untreated fertile males (0 Gy) and males irradiated with 170 and 220 Gy of gamma and X-rays.*

Sterilization doses for genetic sexing and bisexual strains of Anastrepha fraterculus (Morphotype 1)

Anastrepha fraterculus, the South American fruit fly, is a pest that has a significant impact on the fruit industry in South America. The SIT can be used as a supplementary tool in the fight against this pest on an area-wide basis. The development of an artificial rearing system that allows for a rapid colony build-up and the production of larger quantities of sterile flies to meet demands for pilot programmes against this pest has made significant progress. As previously reported, the IPCL has developed a genetic sexing strain (GSS) of *A. fraterculus* based on pupal colour dimorphism, in which adult males emerge from brown pupae while females emerge from black pupae. Females and males can be easily separated using this pupal colour trait, allowing for male-only field releases. This *A. fraterculus* GSS was developed from a laboratory population of the *A. fraterculus* morphotype 1, i.e. the population that is distributed in southern and northern Argentina. Ms Paloma Della Giustina, a fellow from Brazil, has been assisting with an assessment of the effect of radiation on the GSS in comparison with the wild type of strain from Vacaria in southern Brazil. The experiment was carried out under an oxygen-reduced atmosphere, which is a commonly used protocol to protect fruit fly pupae during irradiation, minimizing quality reduction of the sterile male flies.
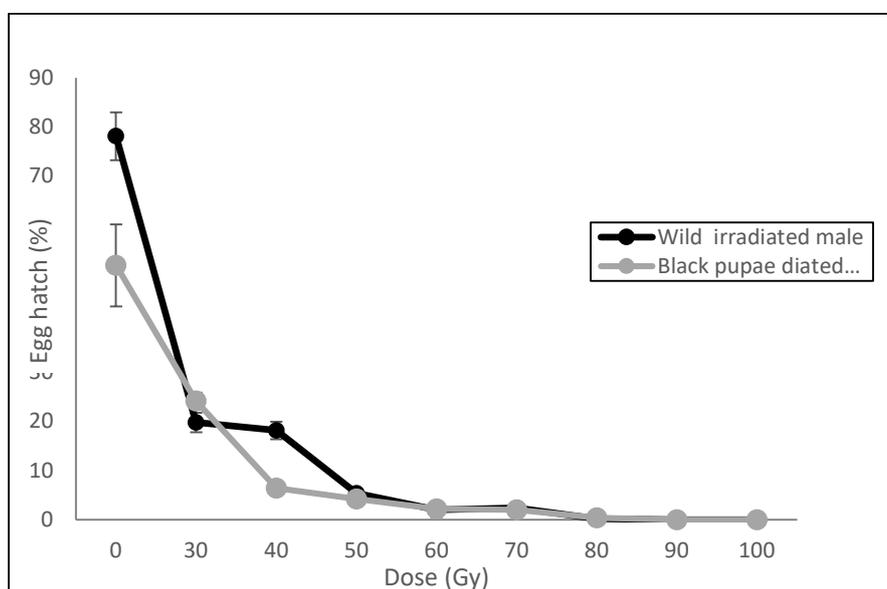
FIG. 17. Dose-response curve for fertility (egg hatch) from crosses between untreated female Anastrepha fraterculus *and males from a genetic sexing strain (GSS) and a bisexual strain (Vacaria) irradiated under hypoxia (Giustina et al., 2021. https://doi.org/10.3390/insects12040308).*

This study tested a range of gamma radiation doses administered to pupae from a bisexual strain and the new GSS under hypoxia. Complete sterilization was achieved with a dose of 80 Gy (Fig. 17). The quality of the sterile insects in terms of adult emergence was similar between strains, but slightly lower in comparison with an untreated control group. This information is therefore valuable for the implementation of SIT against *A. fraterculus* in the region.

CAPACITY BUILDING & SERVICES

In 2021, the IPCL hosted two cost-free experts (CFE) and 15 consultants (C) (of which nine were PhD students), 13 interns, two fellows (F) and one scientific visitor (SV). The fellows were funded by the IAEA'S Department of Technical Cooperation.

Name	Country	Status	Duration	Topic
DA SILVA, Fabio	Brazil	Intern	8 mth	Phytosanitary treatments
SANCHEZ AGUILAR, Jhonatan	Mexico	Intern	2 mth	Characterizing GSS of fruit flies
LIMA, Inajara	Brazil	Intern	2 mth	Phytosanitary treatments
LOYOLA, Renata	Brazil	Intern	3 mth	Phytosanitary treatments
ANDRADE, Jennifer	Brazil	Intern	7 mth	Phytosanitary treatments
AQUINO JARQUIN, Alexis	Mexico	Intern	1 mth	Rearing and evaluation of new medfly genetic sexing strains
TARET, Gustavo	Argentina	C	4 mth	Validation of new medfly genetic sexing strains under mass-rearing conditions
AKKAR DEMIRER, Umran	Turkey	F	4 mth	Training on fruit fly mass rearing of genetic sexing strain for SIT programme
SANCHEZ AGUILAR, Jhonatan	Mexico	C	2 mth	Phytosanitary treatments
LIMA, Inajara	Brazil	C (PhD)	6 mth	Phytosanitary treatments
MIRIERI, Caroline	Kenya	C (PhD)	4 mth	Impact of stress factors on tsetse flies

Name	Country	Status	Duration	Topic
DIENG, Mouhamadou	Senegal	C (PhD)	8 mth	Tsetse symbionts and refractoriness to trypanosome infection
GSTÖTTENMAYER, Fabian	Austria	C (PhD)	1 mth	Development of microsatellite markers for population genetics studies of *Glossina brevipalpis* and *Glossina austeni*
NAWAZ, Arooj	Pakistan	C	7 mth	Maintenance of a tsetse production unit for the support of the tsetse SIT in Senegal
HUDITZ, Hannah	Austria	C(PhD)	5 mth	Analysing the role of Iflavirus and Negavirus presence in tsetse colonies for more cost-effective mass rearing
GSTÖTTENMAYER, Fabian	Austria	Intern	6 mth	Development of microsatellite markers for population genetics studies of *Glossina brevipalpis* and *Glossina austeni*
MENGUAL, Adria	Spain	C	4 mth	Analysing tissue tropism of tsetse symbionts using the fluorescent in situ hybridization (FISH) method

Name	Country	Status	Duration	Topic
M. MAHAMAT, Hissene	Chad	F	4 mth	Evaluation of radiation sensitivity and mating performance of *Glossina fuscipes fuscipes* males
TAQI, Syeda	Pakistan	Intern	12 mth	Development of tsetse SIT packages through colony maintenance
KISWENDA-SIDA DERA, Mikhailou	Burkina Faso	C (PhD)	10 mth	Analysis of tsetse population genetics
SOUKIA, Olga	Greece	Intern	6 mth	Assessing the effect of sorting with the NIRPSS on late-stage tsetse pupae
ISMAIL, Jasmin	Austria	Intern	10 mth	Assessing the impact of aluminium nanoparticles on tsetse symbionts
CLARAMONTE, Carlos	Spain	Intern	1 mth	Assessing the impact of aluminium nanoparticles on tsetse symbionts
RODRIGUEZ, Montse	Spain	Intern	8 mth	Performing population genetics studies of *Glossina brevipalpis* and *Glossina austeni*
ARISTIDE, Kabore	Burkina Faso	C (PhD)	5 mth	Sterilizing effect of X and y̓ rays on tsetse pupae compared

Name	Country	Status	Duration	Topic
BOIKANYO, Salomon	South Africa	SV	2 days	Tsetse rearing procedures review for the support of the tsetse SIT in South Africa
GOUVI, Georgia	Greece	C (PhD)	12 mth	Cytogenetics in tephritid species
SOLLAZZO, Germano	Italy	CFE (PhD)/C(PhD)	12 mth	Temperature-sensitive lethal genes in tephritid species
GRIGORIOU, Maria-Eleni	Greece	CFE/C	12 mth	White pupae gene mutations in *Bactrocera correcta* and *Bactrocera oleae*
VARONE, Marianna	Italy	C	4 mth	Rearing and quality control analysis of mosquito species
IBANEZ, Santiago	Mexico	Intern	4 mth	Detection and characterization of mutations leading to visible phenotypes in *Aedes* species
BIMBILE SOMDA, Severin	Burkina Faso	C	12 mth	Irradiation of mosquitoes, particularly adult irradiation
BOIKANYO, Salomon	South Africa	F	3 mth	

In 2021, the Genetics and Molecular Biology (GMB) group maintained 12 species of fruit flies (134 strains/colonies/populations) and two species of mosquitoes (60 strains/colonies/populations in total). The Plant Pests (PP) group maintained 17 species of fruit flies (68 strains/colonies/populations), the Livestock Pests (LP) group maintained seven tsetse species (eight strains) and the Human Disease Vectors (HDV) group maintained three mosquito species (12 strains).

The GMB and PP groups carried out 68 fruit fly shipments to 23 institutions in 15 countries (Germany, Senegal, Egypt, Kenya, Belgium, France, Italy, Spain, Sweden, Canada, Greece, Netherlands, Mauritius, UK and Croatia), and six shipments of preserved fruit flies to the USA, UK, South Africa, Brazil, Italy

and Belgium. The LP group carried out 116 tsetse shipments of 81,458 pupae (69,773 *G. palpalis gambiensis* pupae to Senegal) to seven institutions in five countries (Senegal, UK, USA, Italy, Australia and the Netherlands). The HDV group carried out 22 mosquito shipments to eight institutions in seven countries (France, UK, South Africa, Italy, Germany, Senegal and Switzerland).

In 2021, the IPCL received 106 visitors from 19 countries.

PUBLICATIONS

ABD-ALLA, A.M.M., M.H. KARIITHI and M BERGOIN. (2021). Managing pathogens in insect mass-rearing for the sterile insect technique, with the tsetse fly salivary gland hypertrophy virus as an example, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 317-354.

ARAÚJO, H.R.C., D.O. CARVALHO and M.L. CAPURRO. (2021) *Aedes aegypti* control programmes in Brazil, In: Hendrichs J., Pereira R. and Vreysen M.J.B., (Eds.), Area-Wide Integrated Pest Management: Development and Field Application, CRC Press, Boca Raton, FL, USA. pp 339-366.

AUGUSTINOS, A.A., G.A. KYRITSIS, C. CÁCERES and K. BOURTZIS. (2021). Insect symbiosis in support of the sterile insect technique, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 605-630.

BAKHOUM, M.T., M.J.B. VREYSEN and J. BOUYER. (2021) The use of species distribution modelling and landscape genetics for tsetse control, In: Hendrichs J., Pereira R. and Vreysen M.J.B., (Eds.), Area-Wide Integrated Pest Management: Development and Field Application, CRC Press, Boca Raton, FL, USA. pp 857-868.

BAKRI, A., K. MEHTA and D.R. LANCE. (2021). Sterilizing insects with ionizing radiation, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 355-398.

BALATSOS, G., A. PUGGIOLI, V. KARRAS, I. LYTRA, J. BOUYER et al. (2021). Reduction in egg fertility of *Aedes albopictus* mosquitoes in Greece following releases of imported sterile males. Insects 2021, 12, 110.

BELLINI, R., M. CARRIERI, F. BALESTRINO, A. PUGGIOLI, J. BOUYER et al. (2021) Field competitiveness of *Aedes albopictus* [Diptera: Culicidae] irradiated males in pilot sterile insect technique trials in northern Italy. Journal of Medical Entomology, Volume 58, Issue 2, Pages 807–813.

BELLO-RIVERA, A., R. PEREIRA, W. ENKERLIN, S. BLOEM, K. BLOEM et al. (2021) Successful area-wide programme that eradicated outbreaks of the invasive cactus moth in Mexico, In: Hendrichs J., Pereira R. and Vreysen M.J.B., (Eds.), Area-wide Integrated Pest Management: Development and Field Application, CRC Press, Boca Raton, FL, USA. pp 561-580.

BENAVENTE-SÁNCHEZ, D., J. MORENO-MOLINA and R. ARGILÉS-HERRERO. (2021) Prospects for remotely piloted aircraft systems in area-wide integrated pest management programmes, In: Hendrichs J., Pereira R. and Vreysen M.J.B., (Eds.), Area-wide Integrated Pest Management: Development and Field Application, CRC Press, Boca Raton, FL, USA. pp 903-916.

BOURTZIS, K., M.J.B. VREYSEN (2021). Sterile Insect Technique (SIT) and its applications. Insects 12, 638.

BOUYER, J., J.ST.H. COX, L. GUERRINI, R. LANCELOT, M.J.B. VREYSEN et al. (2021). Using geographic information systems and spatial modelling in area-wide integrated pest management programmes that integrate the sterile insect technique, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 703-730.

CECILIA, H., S. ARNOUX, S. PICAULT, M. VREYSEN, J. BOUYER et al. (2021). Dispersal in heterogeneous environments drives population dynamics and control of tsetse flies. Proc. R. Soc. B 288: 20202810.

CHAILLEUX, A., D.S. THIAO, S. DIOP, S. AHMAD, C. CACERES et al. (2021). Understanding *Bactrocera dorsalis* trapping to calibrate area-wide management. Journal of Applied Entomology 145(9):831-840.

DE BEER, C.J., A.H. DICKO, J. NTSHANGASE, J. BOUYER, M.J.B. VREYSEN et al (2021). A distribution model for *Glossina brevipalpis* and *Glossina austeni* in Southern Mozambique, Eswatini and South Africa for enhanced area-wide integrated pest management approaches. PLoS Neglected Tropical Diseases. PLoS Neglected Tropical Diseases 15(11):e0009989.

DEMIRBAS-UZEL, G., A.A. AUGUSTINOS, A.G. PARKER, K. BOURTZIS, A.M.M. ABD-ALLA et al. Interactions between tsetse endosymbionts and *Glossina pallidipes* salivary gland hypertrophy virus in heterologous *Glossina* hosts. Frontiers in Microbiology 12:653880.

DIAS V.S., C. CACERES, A. PARKER, R. PEREIRA, U. GULER-DEMIRBAS, A.M.M. ABD-ALLA et al. (2021). Mitochondrial superoxide dismutase overexpression and low oxygen conditioning hormesis improve the performance of irradiated sterile males. Scientific Reports 11:20182.

DOUCHET, L., M. HARAMBOURE, T. BALDET, G. L'AMBERT, J. BOUYER et al. (2021). Comparing sterile male releases and other methods for integrated control of the tiger mosquito in temperate and tropical climates. Scientific Reports 11, 7354.

DROSOPOULOU, E., A. DAMASKOU, A. MARKOU, A. A. AUGUSTINOS, K. BOURTZIS et al. (2021). The complete mitochondrial genomes of *Ceratitis rosa* and *Ceratitis quilicii*, members of the *Ceratitis* FAR species complex (Diptera: Tephritidae). Mitochondrial DNA B 6: 1039-1041.

DYCK, V.A., J. REYES FLORES, M.J.B. VREYSEN, E.E. REGIDOR FERNÁNDEZ, D. LINDQUIST et al. (2021). Management of area-wide pest management programmes that integrate the sterile insect technique, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 781-814.

ENKERLIN, W.R. (2021). Impact of fruit fly control programmes using the sterile insect technique, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 979-1006.

FELDMANN, U., V.A. DYCK, R.C. MATTIOLI, J. JANNIN and M.J.B. VREYSEN. (2021). Impact of tsetse fly eradication programmes using the sterile insect technique, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 1051-1080.

FRANZ, G., K. BOURTZIS and C. CÁCERES. (2021). Practical and operational genetic sexing systems based on classical genetic approaches in fruit flies, an example for other species amenable to large-scale rearing for the sterile insect technique, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 575-604.

GATO, R., Z. MENENDEZ, E. PRIETO, R. ARGILES, J. BOUYER, et al. (2021). Sterile insect technique: successful suppression of *Aedes aegypti*. Field Population in Cuba. Insects 12, 469.

GIMONNEAU, G., R. OUEDRAOGO, E. SALOU, J.B. RAYAISSE, J. BOUYER et al. (2021) Larviposition site selection mediated by volatile semiochemicals in *Glossina palpalis gambiensis*. Ecological Entomology, 46: 301-309.

GIUSTINA, P.D., T. MASTRANGELO, S. AHMAD, G. MASCARIN, C. CACERES. (2021) Determining the sterilization doses under hypoxia for the novel black pupae genetic sexing strain of *Anastrepha fraterculus* (Diptera, Tephritidae). Insects, 12, 308.

GÓMEZ-SIMUTA Y., A. PARKER, C. CÁCERES, M.J.B. VREYSEN, H. YAMADA (2021). Characterization and dose-mapping of an X-ray blood irradiator to assess application potential for the sterile insect technique (SIT), Applied Radiation and Isotopes, 176, 109859,

HÄCKER, I., K. BOURTZIS and M.F. SCHETELIG. (2021). Applying modern molecular technologies in support of the sterile insect technique, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 657-702.

HENDRICHS, J. and A.S. ROBINSON. (2021). Prospects for the future development and application of the sterile insect technique, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 1119-1170.

HENDRICHS, J., M.J.B. VREYSEN, W.R. ENKERLIN and J.P. CAYOL. (2021). Strategic options in using sterile insects for area-wide integrated pest management, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 841-884.

KLASSEN, W. and M.J.B. VREYSEN. (2021). Area-wide integrated pest management and the sterile insect technique, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 75-112.

KOSKINIOTI, P., A.A. AUGUSTINOS, D.O. CARVALHO, R. ARGILES-HERRERO, K. BOURTZIS et al. (2021). Genetic sexing strains for the population suppression of the mosquito vector *Aedes aegypti*. Philosophical Transactions Royal Society B 376:20190808.

LEES, R.S., D.O. CARVALHO and J. BOUYER. (2021). Potential impact of integrating the sterile insect technique into the fight against disease-transmitting mosquitoes, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 1081-1118.

LI, Y., L.A. BATON, D. ZHANG, J. BOUYER, A.G. PARKER et al. (2021). Reply to: Issues with combining incompatible and sterile insect techniques. Nature 590, E3–E5.

MAMAI, W., H. MAIGA, N.S. BIMBILE SOMDA, T. WALLNER, O.B. MASSO, H. YAMADA, J. BOUYER et al. (2021). Does tapwater quality compromise the production of *Aedes* mosquitoes in genetic control projects? Insects 12, 57.

MANGAN, R.L. and J. BOUYER. (2021). Population suppression in support of the sterile insect technique, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 549-574.

MARINA, C.F., J.G. BOND, K. HERNÁNDEZ-ARRIAGA, D.O. CARVALHO, K. BOURTZIS et al. (2021). Population dynamics of *Aedes aegypti* and *Aedes albopictus* in two rural villages in southern Mexico: baseline data for an evaluation of the sterile insect technique. Insects 12, 58.

MASTRANGELO, T., A. KOVALESKI, B. MASET, M.D.L.Z. COSTA, C. CACERES et al. (2021). Improvement of the mass-rearing protocols for the south american fruit fly for application of the sterile insect technique. Insects 12, 622.

MEKI I.K., H.I. HUDITZ, A. STRUNOV, R. VAN DER VLUGT, A.M.M. ABD-ALLA et al (2021). Characterization and tissue tropism of newly identified iflavirus and negevirus in tsetse flies *Glossina morsitans morsitans*. Viruses 13, 2472.

NIKOLOULI, K., F. SASSU, C. STAUFFER, C. CÁCERES, K. BOURTZIS et al. (2021). Enterobacter sp. AA26 as a protein source in the larval diet of *Drosophila suzukii*. Insects 12, 923.

OLIVA, C.F., M.Q. BENEDICT, C.M. COLLINS, T. BALDET, J. BOUYER et al. (2021). Sterile Insect Technique (SIT) against *Aedes* species mosquitoes: A roadmap and good practice framework for designing, implementing and evaluating pilot field trials. Insects 12, 191.

PARKER, A.G., M.J.B. VREYSEN, J. BOUYER and C.O. CALKINS. (2021). Sterile insect quality control/assurance, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 399-440.

PARKER, A.G., W. MAMAI and H. MAIGA. (2021). Mass-rearing for the sterile insect technique, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 283-316.

RAMIREZ-SANTOS, E.; P. RENDON, G. GOUVI, K. BOURTZIS, C. CACERES et al. (2021). A novel genetic sexing strain of *Anastrepha ludens* for cost-effective sterile insect technique applications: improved genetic stability and rearing efficiency. Insects 12, 499.

ROBINSON, A.S. (2021). Genetic basis of the sterile insect technique, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 143-162.

SASSÙ, F., T. BAKHOUM, J. BOUYER, C. CÁCERES (2021). Mating competitiveness of sterile male *Drosophila suzukii* under different atmosphere conditions. Proceedings 68.

SASSÙ, F., K. NIKOLOULI, C. STAUFFER, K. BOURTZIS and C. CÁCERES-BARRIOS. (2021) Sterile insect technique and incompatible insect technique for the integrated *Drosophila suzukii* management. In: Flávio Roberto Mello Garcia (ed.), *Drosophila suzukii* management. Springer. pp 169-194.

SAVINI, G., F. SCOLARI, L. OMETTO, O. ROTA-STABELLI, A. M. M. ABD-ALLA et al. Viviparity and habitat restrictions may influence the evolution of male reproductive genes in tsetse fly (*Glossina*) species. BMC Biol 19, 211.

SON J.H., B.L. WEISS, Ks. M. DERA, F. GSTOTTENMAYER, A.M.M. ABD-ALLA et al. (2021). Infection with endosymbiotic *Spiroplasma* disrupts tsetse (*Glossina fuscipes fuscipes*) metabolic and reproductive homeostasis. PLoS Pathogy 17(9), e1009539.

TAIT G., S. MERMER, D. STOCKTON, J. LEE, F. SASSU et al. (2021). *Drosophila suzukii* (Diptera: Drosophilidae): A decade of research towards a sustainable integrated pest management program. Journal of Economic Entomology, 114(5), 1950–1974.

VILJOEN, G.J., R. PEREIRA, M.J.B. VREYSEN, G. CATTOLI, M. GARCIA PODESTA (2021). Agriculture: improving livestock production, In: Greenspan E. (Ed.), Encyclopedia of Nuclear Energy, Elsevier, Amesterdam, Netherlands. Vol.4, pp. 302-312.

VREYSEN, M.J.B. (2021). Monitoring sterile and wild insects in area-wide integrated pest management programmes, In: Dyck V.A., Hendrichs J. and Robinson A.S., (Eds.), Sterile Insect Technique: Principles

and Practice in Area-Wide Integrated Pest Management, 2nd ed., CRC Press, Boca Raton, FL, USA. pp 485-528.

VREYSEN, M.J.B., A.M.M. ABD-ALLA, K. BOURTZIS, J. BOUYER, C. CACERES, C. DE BEER, D. OLIVEIRA CARVALHO, H. MAIGA, W. MAMAI, K. NIKOLOULI, H. YAMADA, and R. PEREIRA. (2021). The Insect pest control laboratory of the joint FAO/IAEA programme: ten years (2010–2020) of research and development, achievements and challenges in support of the sterile insect technique. Insects, 12, 346.

VREYSEN, M.J.B., M.T. SECK, B. SALL, A.G. MBAYE, J. BOUYER et al. (2021). Area-wide integrated management of a *Glossina palpalis gambiensis* population from the Niayes area of Senegal: A review of operational research in support of a phased conditional approach, In: Hendrichs J., Pereira R. and Vreysen M.J.B., (Eds.), Area-wide Integrated Pest Management: Development and Field Application, CRC Press, Boca Raton, FL, USA. pp 275-304.

WARD, C., K. NIKOLOULI, G. GOUVI, C. CÁCERES-BARRIOS, K. BOURTZIS et al. (2021). White pupae phenotype of tephritids is caused by parallel mutations of a MFS transporter. Nature Communications 12:491.

ZHANG D., S. CHEN, A.M.M. ABD-ALLA, K. BOURTZIS (2021). The effect of radiation on the gut bacteriome of *Aedes albopictus*. Fro

THE PLANT BREEDING AND GENETICS LABORATORY

EXECUTIVE SUMMARY

The Plant Breeding and Genetics Laboratory (PBGL) of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture assists Member States (MSs) in the development and transfer of nuclear and related (bio)technologies for mutation-assisted breeding to improve crops for transboundary plant pest and disease resistance, better adaptation to climate change, and improved food security.

In terms of plant health, food security and climate change adaptation, the PBGL actively worked to support MSs in addressing the Fusarium wilt disease in banana, leaf rust in arabica coffee and the parasitic weed *Striga* in sorghum. These pests and diseases result in huge economic losses for the affected MSs. In 2021 the Fusarium wilt strain Tropical Race 4 (TR4) was confirmed in Peru, which marked the second report of TR4 in Latin America after Colombia confirmed the first TR4 occurrence in 2019. The presence of TR4 within Latin America threatens the global Cavendish banana production and exportation market which is primarily centred in this region. Coffee leaf rust (CLR) is a major production constraint in arabica coffee plantations in Central and South America and parts of Africa. Coffee leaf rust is exacerbated by climate change further spreading this disease to previously unaffected areas. The economic losses attributed to the parasitic weed *Striga* in cereals in Sub-Saharan Africa alone amount to USD 7 billion annually, according to FAO. *Striga* particularly causes productivity losses in agricultural systems with limited resources.

Recent interest in the use of mutation breeding for the control of banana Fusarium wilt by MSs in Africa and Latin America led to an increase of research activities in banana. In 2021 the PBGL adapted *in vitro* micropropagation, ethyl methane sulfonate (EMS) and gamma-ray irradiation protocols for the first time to an edible diploid (AA) banana called Mchare, an important staple and source of income in Eastern Africa. Importantly, Mchare is at the origin of the triploid Cavendish (AAA) banana which is now under threat by TR4 globally. Using inoculation studies, Mchare was shown to be highly susceptible to TR4. Over 4,000 *in vitro* Mchare plantlets have been produced for bulk irradiation and subsequent field-based Fusarium wilt resistance screening in Tanzania planned for 2022.

Other important work conducted on plant pests and diseases in 2021 were the validation and transfer of the bioassay for quantitative analysis of the *Striga* resistance/tolerance mechanism to Burkina Faso. Using this bioassay, four distinct *Striga* resistance mechanisms could be identified in mutant sorghum lines produced under the coordinated research project (CRP) D25005. This knowledge can guide further breeding work in Burkina Faso and Sudan and molecular genetic studies towards identifying the causative mutations underlying the *Striga* resistance mechanisms.

The PBGL also made significant progress in 2021 in applying bioinformatics and molecular genetics tools for accelerated breeding. The laboratory's computational workflow for whole-genome sequence analysis was integrated with the publicly available software tools QTL-BSA and CNV-seq for genetic mapping and detection of copy number variation, respectively. Using QTL-BSA, the causative mutation(s) underlying the gamma-ray induced semi-dwarf/early maturity trait in sorghum was confirmed to be located on chromosome 4 near the centromere. Using CNV-seq, 28 large deletions with sizes between 0,1 and 1 Mb could be identified in four gamma-ray irradiated mutant Cavendish lines.

In 2021, a second genotyping platform based on KASP™ (Kompetitive Allele Specific Polymerase Chain Reaction) was established at the PBGL and successfully used to validate one KASP™ marker for

Marker-Assisted Backcrossing (MAB) of the early maturity/semi-dwarf trait in sorghum for improved lodging resistance and terminal drought tolerance. Similarly, one KASP™ marker was validated for MAB of a feed quality trait in barley.

The PBGL's molecular marker and bioinformatics tools are broadly applicable to seed crops and can be rolled out in 2022 for use by plant breeders across Africa, Asia, Europe, and Latin America, as already shown in the barley breeding program of Austria for marker-assisted pyramiding of two mutant traits for improved feed quality.

In 2021, PBGL pioneered two laboratory procedures for mutation detection at population scale. In one method polymerase chain reaction (PCR) amplicons were sequenced in arabica coffee and banana using Oxford Nanopore long read sequencing and a hand-held sequencer that is run on a laptop. A second approach involves the use of digital PCR, an ultrasensitive mutation detection method applied in medical diagnostics. The PBGL is adapting this technology for detection of single nucleotide variants (SNV) in mutant populations. In 2021, a SNV detection limit of ca 0.17% could be achieved in sorghum, indicating significantly increased throughput and precision compared to existing mutation detection technologies.

Capacity building and knowledge dissemination has been central to the activities of the PBGL through (a) published protocols/technical guidance for MSs: 11 publications (co-)authored by the PBGL staff including one book, five book chapters and one protocol plus technical guide on MAB; (b) drafting of three CRP protocol books as outputs of CRP D22005 and CRP D25005: (i) 'Efficient Screening Techniques for Banana Fusarium TR4 Resistance': 11 protocols finalized with book submitted to Springer Nature; (ii) 'Mutation Breeding in Coffee for Leaf Rust resistance': 15 protocols drafted; (iii) 'Mutation Breeding in Cereals for Resistance to the Parasitic Weed *Striga*': 10 protocols drafted; (c) resource mobilization to enhance capacity building and technology transfer in banana and coffee mutation breeding for East Africa with financial support from Belgium (IAEA Peaceful Uses Initiative).

Four interns, one fellow and one PhD student were trained at the PBGL in the use of nuclear and related biotechnology and bioinformatics techniques for crop pest and disease resistance and food security. The PBGL staff presented 17 webinars in a virtual training course on mutation breeding, attended by 51 participants from 34 European and African MSs.

In terms of crop irradiation services to MSs, the PBGL mutagenized 297 samples for 25 different MSs in 2021, compared to 124 samples from 19 MSs in 2020, as the COVID-19 restrictions for international shipments have been relaxed in 2021. Most treatments were carried out in support of Technical Cooperation and CRP projects.

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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Plant Health and Food Security

Adapting mutation induction techniques to African diploid cooking banana for Fusarium wilt resistance

Mchare (AA) cooking bananas are a vital food and income-generating crop to people in northern Tanzania, the neighbouring countries, and islands off the east coast of Africa. Mchare are a distinct group of edible AA diploid bananas, and the unreduced gamete parent of the triploid AAA Cavendish, Gros Michel and many other dessert bananas. Mchare production is, however, constrained by various pests and diseases, including Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Foc). The only means to deal with Fusarium wilt in East Africa is to prevent the introduction of the fungus into banana fields, and the replacement of susceptible with resistant varieties.

Conventional banana breeding is slow due to the long-life cycle, sterility and large plant size. About 20 years are needed to breed improved Matooke and plantain hybrids. Hence *in vitro* tissue culture, combined with induced mutagenesis, could substantially reduce the time to develop Fusarium wilt-resistant banana cultivars while retaining the existing valuable characteristics such as taste, bunch size and cycle time. Hence, a major objective of the PUI 'Enhancing climate change adaptation and disease resilience in banana-coffee cropping systems in East Africa', is to produce Mchare banana mutant lines that are resistant to Fusarium using mutation-assisted breeding.

In 2021, about 1,500 *in vitro* plantlets of two Mchare varieties were shipped from the International Institute of Tropical Agriculture (IITA), Tanzania to the PBGL. The plantlets were subcultured once in semi-solid medium. Healthy plantlets were next moved to *in vitro* liquid media for establishment and multiplication. Protocols for *in vitro* micropropagation and mutation induction of triploid Cavendish bananas were adapted for first time to an edible diploid (AA) banana Mchare. Results for gamma-ray mutagenesis radio-sensitivity testing of Mchare cv Mshale (ITC1223) are presented in FIG 1. Overall, based on two different growth parameters measured, an LD_{30} of ca. 10 Gy was determined.
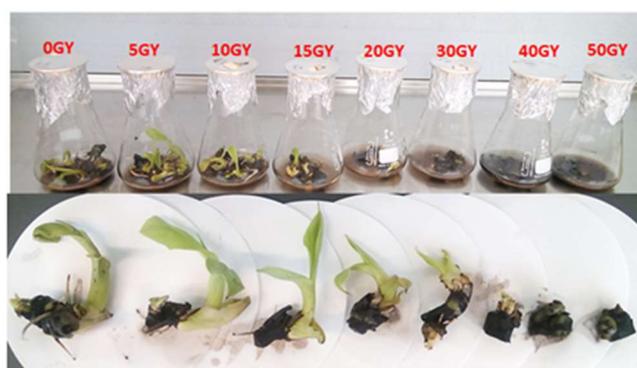
FIG. 1 Response of in-vitro Mchare banana plantlets to different Gamma dose and control. From left to right: 0, 5, 10, 15, 20, 30, 40 and 50 Gy.

Similarly, an EMS kill curve was established for shoot tips of Mchare cv Mshale in replicated experiments using four shoot tips per treatment as illustrated in FIG 2. Based on two different growth parameters measured, an LD_{30} of ca 1,0 % was estimated.
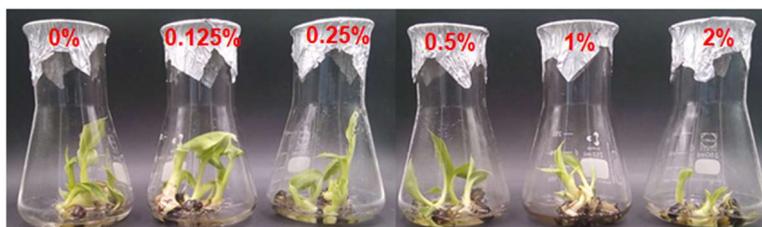
FIG. 2 Response of in-vitro Mchare banana plantlets to different EMS doses and control. From left to right: 0%,0.125%, 0.25%, 0.5%, 1%, and 2%.

Mchare is susceptible to Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) race 1. Using inoculation studies conducted at the PBGL, Mchare was shown to be highly susceptible to Foc Tropical Race 4 as shown in FIG 3.

FIG. 3 Response of Mchare banana plantlets to inoculation with Fusarium wilt tropical Race 4, two inoculated plants on the left, compared to uninoculated plants on the right, four weeks after inoculation.

Distinct *Striga* resistance mechanisms identified in advanced mutant sorghum lines using a lab-based bioassay

Under the *Striga* CRP D25005, the PBGL conducted further experiments for identification of the mechanism of resistance in eight, gamma-induced, sorghum mutants with verified resistance to *Striga* hermonthica from Burkina Faso. These mutants were induced in three farmer preferred varieties of sorghum (Sa-P, GK-P, and IC-P) each had two, three and three mutants, respectively. Seeds from each of the mutants and parents were surface sterilized and germinated in petri-dishes on moist filter paper and placed in an incubator under 28°C at 16/8 hr light/dark regime. Extended gel and mini-rhizotron assays were conducted to assess *Striga* germination rate, post germination attachment and establishment of parasitism on resistant mutants and wild parents following protocols adapted at the PBGL. For the extended gel assay, the relative germination percentage of resistant versus wild type plants was determined: three replicates each with three plates were assigned to each genotype (see FIG. 4). Data were compiled and statistically analyzed using Duncan's Multiple Range Test at 0.001 probability. The extended gel assay experiments indicated that three of the mutants (one from each parent) have a low rate of *Striga* germination due to either low stimulant production or the presence

of a germination inhibitor. The mini-rhizotron assay is based on growing seven-day old sorghum seedling in a petri-dish (90 cm Ø) filled with fine sand and covered with filter paper. The seedling was gently placed on the surface and surrounded by preconditioned *Striga* seeds. Mini-rhizotrons were incubated at 28°C under a 16/8 hr light/dark regime. The cultures were inspected weekly for up to one month for *Striga* seed germination, haustoria development and attachment, necrosis, and growth rate of *Striga* plants. The results confirmed the low germination rate of *Striga* and/or presence of germination inhibitor observed in the gel assay. In addition, some mutants showed a hypersensitive reaction and necrosis killing the Striga parasite or limiting its growth rate compared to the wild parents (see FIG. 5). Seven of the eight mutants had a significantly lower number *Striga* plants attached to the host root compared to the wild parent at 30 days. Genomic DNA from three sorghum mutants and parents together with bulks of sensitive siblings was sent for whole genome sequencing and will be analyzed for discovery of the possible causative mutations for the resistance to *Striga hermonthica*.

FIG. 4. Representative extended gel-assay for assessment of induced germination rate of Striga seeds among mutants and parents. From left to right: whole plate showing the position of select areas for data collection, and the two selected areas magnified for counting germinating Striga seeds.

FIG. 5. Representative mini-rhizotron assays showing from left to right: early normal attachment; healthy Striga; Striga necrosis; hypersensitive response.

Breeding technologies and bioinformatics

Decreasing costs and improved sequencing technologies along with new genotyping methods are revolutionizing the way plant breeding is practiced. These tools also offer unprecedented opportunities to add precision and fast track plant mutation breeding programs. Comparing, analyzing, and visualizing induced variation in plants requires specialized computational tools and strategies. PBGL strives to enable MSs to fully participate in these advanced technologies and so enhance their mutation breeding projects.

Marker-Assisted Backcrossing (MAB) protocols for accelerated breeding of mutant traits

MAB allows accurate and efficient introgression of important agronomic trait(s) from a germplasm source to desired elite lines without going through the time-consuming phenotypic selection. MAB is particularly applicable to induced mutant traits which are predominantly monogenic and recessive. A breeder-friendly marker kit and MAB protocol for improved feed quality in barley was published in 2021. The publication provides laboratory and greenhouse protocols for the development and application of a SNP marker assay using standard molecular biology techniques and infrastructures. It introduces methods for plant genotyping, provides guidance for PCR primer design and for chi-square goodness of fit analysis of genotypes. The protocols and principles described in this booklet can be broadly applied to any Single Nucleotide Polymorphism or small Insertion/Deletion (INDEL) in seed-propagated crops.

A Low-Cost Genotyping Protocol and Kit for Marker-Assisted Selection of Orange Lemma (*rob1.a*), a Feed Quality Trait in Barley (*Hordeum vulgare* L.)

INTRODUCTORY GUIDE AND LABORATORY PROTOCOLS

Photo

Barley field, Raasdorf Experimental Station Groß-Enzersdorf, BOKU-University of Natural Resources and Life Sciences, Vienna, Austria

Joint FAO/IAEA Division,
Plant Breeding and Genetics Laboratory,
Seibersdorf, Austria

A new KASP^{TM} marker for a Gamma-ray Induced Early-Maturing/Semi-Dwarf Trait in African Sorghum

An early-maturing semi-dwarf trait was induced by gamma-ray irradiation of a farmer-preferred sorghum variety (Wad Ahmed) from Sudan. The early maturity trait is useful for drought escape in areas prone to terminal drought while the semi-dwarf trait is useful to improve lodging resistance in high-input agriculture production, besides its suitability for combine harvesting in large-scale mechanized agricultural systems. Previous genetic studies showed that the mutation is controlled by a single recessive gene.

Previously, mapping populations were developed by crossing the mutants with wild parent and F_2 populations were planted in the field and the glasshouse at the PBGL. Comparing genome sequence of the contrasting bulks with short versus tall phenotypes of the mutant and wild parents positioned the causative mutation on chromosome 4, near the centromere. Seven sequence polymorphisms were identified in that region between the mutant versus wild type parent. Two SNPs were analyzed for their potential use as markers for accelerated breeding. Overall, both SNPs were found to be consistent among sibling mutants (D2 and D3) compared to the wild parent producing robust PCR amplicons of the expected size across all genotypes tested. Next, a fluorescence based KASP^{TM} assay was commissioned for further validation and marker-trait association studies at the PBGL.

The KASP^{TM} assays clearly separated individuals from F_2 populations generated by backcrossing the mutant to its wild parent Wad Ahmed showing the homozygous mutants, heterozygous and homozygous wild parent phenotype/genotype alleles in the expected ratios (see FIG. 6). Genotyping the F_2 individuals from two populations (D2 and D3) revealed percent of crossing over between the marker and the trait in two populations in the range of 3–5%. This indicates that the KASP^{TM} markers are closely linked to the mutant trait (3–5 cM) and therefore have the potential to be useful for accelerated transfer of the early maturing/semi-dwarf mutant trait to improve tall varieties and landraces from Member States through Marker-Assisted Backcrossing (MAB).

The KASP^{TM} assays were next validated on crosses involving the mutants and three tall, locally adapted sorghum land races from Africa. One KASP^{TM} marker was perfectly linked with the dwarf allele in the segregating F$_2$ populations with the heterozygous individuals clearly separated from the homozygous wild type, as required in a backcrossing breeding process (See FIG. 7 and FIG. 8).
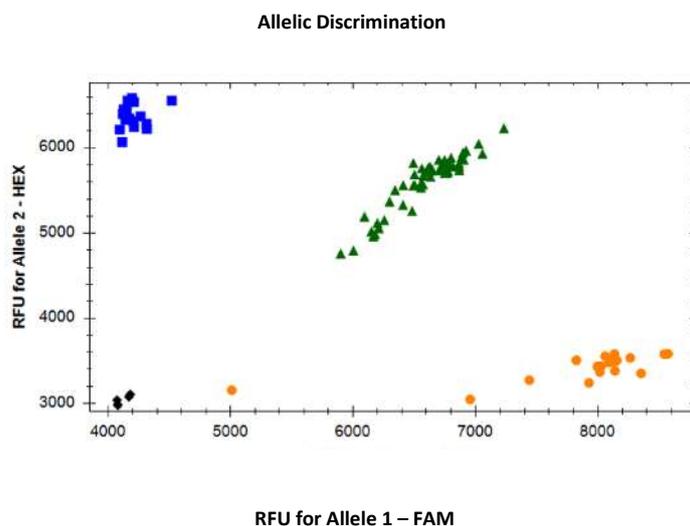
FIG. 6. Representative genotyping image of F2 population (D3 X wild parent) by KASP^{TM} marker for SNP 37 showing marker-trait association with clustering of homozygous individuals for mutant allele (blue), heterozygous (green) and homozygous wild type individuals (orange).
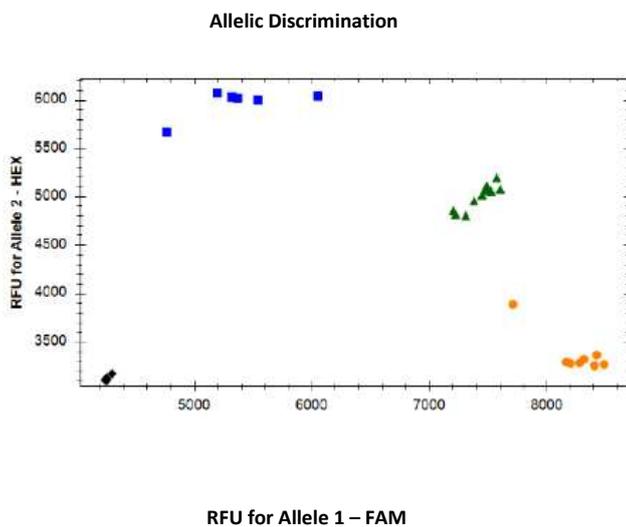
FIG. 7. Representative validation of the KASP marker in one of the three tested landraces whereby the tested F2 individuals are separated by the marker into the three expected groups: homozygous mutant (blue); heterozygous (green) and homozygous wild type (orange).

FIG. 8. Picture showing the tall African sorghum land races with the early maturing-semi-dwarf mutant in the centre used for introgression of the early maturity/semi-dwarf trait and the marker-trait association studies.

Cost-effective mutation detection in coffee and banana with a hand-held DNA sequencer

A significant invention of the past decade has been nanopore sequencing: Nucleotide sequence, DNA or RNA, is determined directly as the single-strand molecules are pulled through protein pores across a membrane. Sequencing read length is limited by the respective molecule and not by the technology. This allows for long, up to mega base pair-long sequencing reads.

Most importantly, this sequencing is possible with a portable, hand-held sequencing device: the 'MinION' sequencer made by Oxford Nanopore Technologies. The 'MinION' has the size of a candy bar and can be operated from a laptop via USB. The consumables are ready-to-use, so-called 'flow cells'. The MinION sequencing 'machine' itself is essentially free with negligent delivery cost and zero set-up and maintenance costs. It can, in principle, be operated by anyone, everywhere and could become a game changer in plant breeding, putting genomics at the fingertips of our partners in Member States.

The MinION technology is still evolving with ever decreasing error rates but has now matured to a point where PBGL has identified PCR amplicon sequencing as an important first application towards mutation detection. Key will be to develop protocols for its cost-effective use at the population-scale, as required in plant (mutation) breeding applications.

In a first pilot we have screened target genes in mutant populations of coffee and banana (FIG 9). The entire workflow can be completed within 24h. Individual samples in the pools are labelled and identified by attaching unique DNA sequences, so-called molecular barcodes. Barcoding allows for pooling many samples into the same sequencing run, greatly reducing cost per sample. PBGL is currently evaluating several published protocols to prepare barcoded amplicons for Nanopore sequencing intended to increase existing barcoding capacities. The goal is to have a robust molecular biology protocol and sequence analysis software pipeline for cost-effective mutation detection at the population scale.
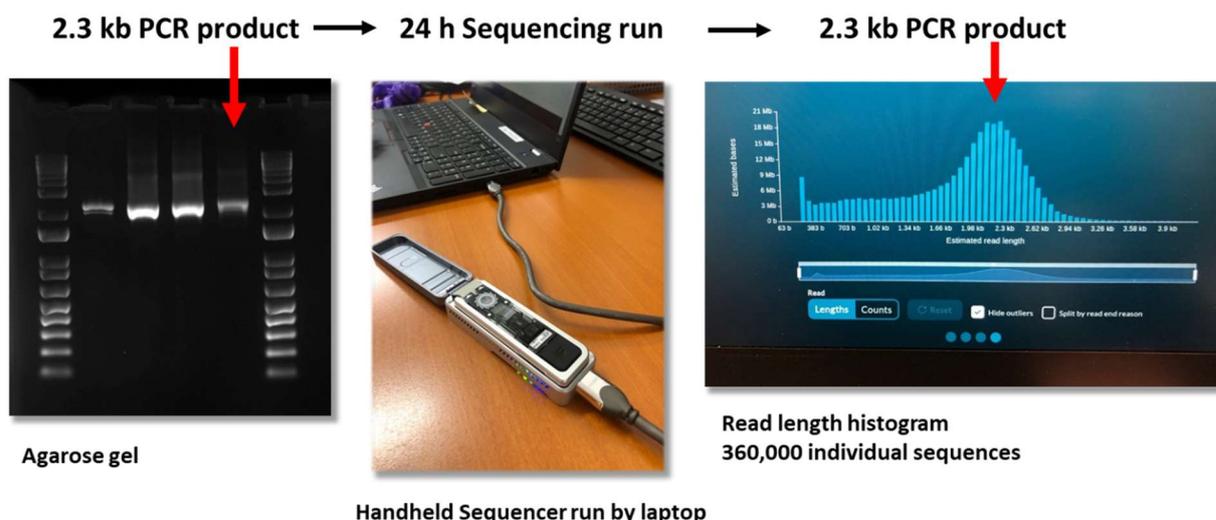
FIG. 9. (left) pools of PCR products for sequencing. (middle) the MinION DNA sequencer loaded with a Flongle flow cell and run by a laptop via USB. (right) read length histogram producing hundreds of thousands individual sequences that can then be analysed to find novel mutations.

Droplet PCR enables rare mutation detection in sorghum

Digital droplet PCR (ddPCR) is a breakthrough technology that offers ultrasensitive and reproducible nucleic acid quantification and detection. The technology is applied in cancer diagnostics for detection of rare somatic variants and copy number variations. In ddPCR, each PCR sample is partitioned into a large number of microscopic droplets prior to amplification. Each droplet is an individual PCR reaction. To report target amplification, specific probes labelled with fluorophores FAM and/or HEX can be used. After amplification, fluorescence is detected in each droplet in which the target sequence was amplified. Droplet without the target sequence show little or no fluorescence and are scored as negative. The fraction of positive droplets is converted to the number of molecules in the starting sample. A two-day training was organized for the Bio-Rad QX200 ddPCR system in 2021 for PBGL staff. The system includes a droplet generator and a droplet reader (FIG.10). The training was focused on two main ddPCR applications relevant for mutation breeding: detection of rare mutations and copy number variations.

FIG. 10. (left) ddPCR reader and generator. (right) Training of PBGL staff in digital PCR by a BioRad Field Application Specialist Dr P. Scheu, Germany

A pilot project was set up in sorghum to develop a mutant population and screen this population for mutations in trait control genes. Targeted traits/genes include tolerance to the parasitic weed *Striga*

and improved feed quality. In 2021, two probes were designed containing previously characterized SNP mutations. These were used in ddPCR experiments to determine the lower limit of mutant SNP detection using genomic DNA of wild type (WT) sorghum mixed with mutant sorghum DNA (Mt) in different ratios ranging from 50% to 0% Mt DNA. Samples were analysed for the presence of the mutant and wild type allele. So far, mutant SNP detection level has reached ca 0.17 % with further improvement possible (FIG. 11). This result indicates that significant increases in throughput and cost savings can be achieved for targeted SNP detection compared to established technologies such as TILLING.

FIG 11. Representative plot showing number of mutant (bleu) and wild type (brown) genome copies in serial dilutions from left to right; 50% (A), 10% (B), 1% (C), 0.1% (D), and 0% mutant DNA

To successfully apply this technology for mutation breeding, large mutant populations are required to have sufficient statistical probability that a mutation is induced in a particular gene or nucleotide. In 2021 PBGL evaluated seeding and establishment rates of gamma- and X-ray irradiated and control sorghum plants in field experiments at the IAEA experimental farm in Seibersdorf Austria enabling improved high-density sorghum cultivation.

Genomics Applications – genetic mapping and large copy number variants

The low cost of Next Generation Sequencing technologies (NGS) allows researchers around the world to sequence entire genomes. Being able to efficiently associate desired phenotypes -the traits- with differences in the underlying genes can accelerate the breeding process and shorten breeding cycles. PBGL has been using NGS-enabled genomics approaches for a several years now and has developed an analysis pipeline in form of a software workflow, which is publicly available. This initial analysis of the raw NGS data produces lists of observed DNA variations. This information is then applied in the context of the breeding task at hand.

One such application is the genetic mapping of variants that are genetically linked to a trait of interest. Once identified, such linked variants can then be turned into markers for Marker-Assisted Breeding (MAB). A cost-effective approach for genetic mapping is Bulked Segregant Analysis pioneered in the 1990s by R. Michelmore and colleagues[*]. Bulked segregant analysis became more powerful when it was combined with NGS starting in 2009 (by K. Schneeberger and colleagues)[†]. With NGS, all segregating markers could be detected in the process and the allele frequencies in the bulks

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[*] Michelmore RW, Paran I, Kesseli RV, Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA. (1991) 1;88(21):9828-32. doi: 10.1073/pnas.88.21.9828.

[†] Schneeberger, K., Ossowski, S., Lanz, C. *et al.* SHOREmap: simultaneous mapping and mutation identification by deep sequencing. *Nat Methods* **6,** 550–551 (2009). https://doi.org/10.1038/nmeth0809-550

at each marker readily estimated. Different software tools and statistics are available to conduct the mapping from NGS data. PBGL has identified analysis tools that produce meaningful results in our mutation breeding projects and adapted them to integrate well with the output of our variant calling workflow. We are disseminating this procedure in training courses and a step-by-step manual. Figure 12 shows a plot of allele frequencies in the contrasting bulks of an F2 population in sorghum segregating for a semi-dwarf/early maturity trait, showing that chromosome 4 harbours the causative mutation(s) for this trait.
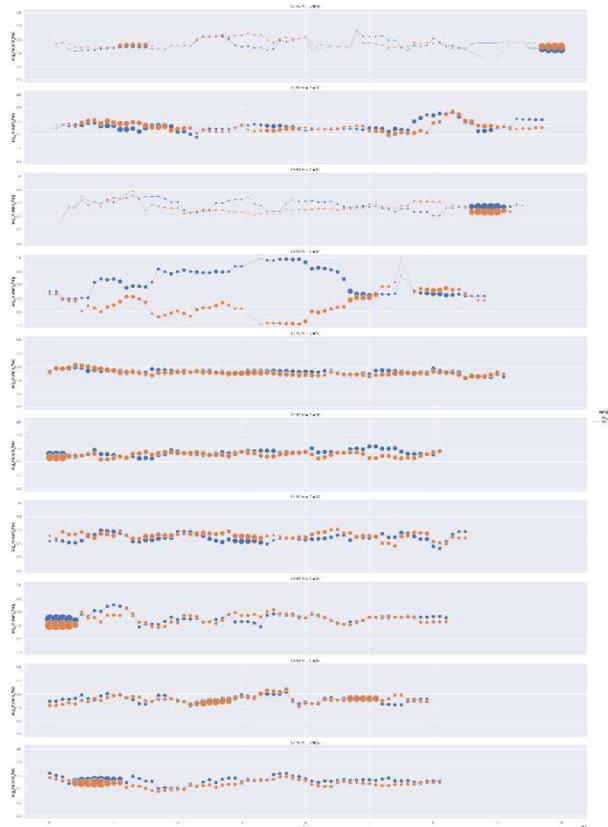
FIG. 12. Allele frequency plot along the 10 chromosomes of sorghum (top is chr 1, bottom is chr 10) derived from bulked F2 progenies with contrasting phenotypes showing candidate region harbouring the causative mutation(s) on chr 4

Another application of NGS data for mutation breeding is the detection of copy number variants, i.e., large deletions and duplications. These are of high interest in mutation breeding particularly after gamma-ray mutagenesis, known to induce large deletions/insertions. Of particular interest is the comparison between mutant and progenitor to identify those copy number variants that arose through the mutagenic treatment. While not readily detected by standard variant callers, the necessary data to detect those is produced in the process: large stretches of DNA that are missing or duplicated will be reflected in the read coverage of alignments of the sequencing reads to the reference genome. A sequencing coverage lower than expected will reveal a deletion, higher than expected indicates a region that is present more often than expected. We identified software (CNV-seq)[*] that implements a statistic for a pairwise comparison of two samples. It allows for comparing mutants with the progenitor and detect statistically significant differences in coverage. An example

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[*] Xie, C., Tammi, M.T. CNV-seq, a new method to detect copy number variation using high-throughput sequencing. *BMC Bioinformatics* **10,** 80 (2009). https://doi.org/10.1186/1471-2105-10-80

is depicted in Figure 13, showing a 6 Mb deletion on chromosome 9 in one of PBGL's sorghum mutants. We have implemented an R version of CNV-seq and guide for its use with a step-by-step manual.
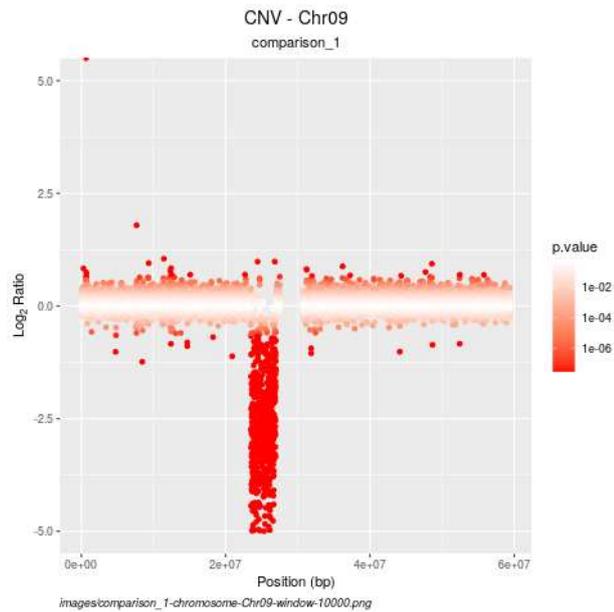
FIG. 13. Detection of a large deletion on chromosome 9 in a Sorghum mutant using and R implementation of CNV-seq (Xie, C et al., 2009) in a Jupyter notebook.

Software Tools Developed by PBGL in 2021

- pbgl-qtl-bsa - Plotting allele frequencies from VCF files (Jupyter/Python);

- pbgl-cnvseq - Identifying copy number variations from BAM files (Jupyter/R);

- pbgl-cnvkit - Identifying copy number variations from BAM files for exome capture data (Jupyter/Python);

- pbgl-gdd - Visualizing INFO and FORMAT attributes from VCF files (Jupyter/Python).

CAPACITY BUILDING & SERVICES

Capacity building

In 2021, the PBGL hosted one PhD student (supported by Belgium through IAEA's Peaceful Uses Initiative), one fellow (F; supported by the IAEA's Department of Technical Cooperation) and four interns (I; the two data science interns were supported by the USA) in the following areas:

Name	Country	Status	Topic	Period
Mr Anibal E. MORALES ZAMBRANA	USA	I	Data science	9 months
Ms Faith LUVAI	Kenya	I	Banana Fusarium wilt	11 months
Mr Hassan MDUMA	Tanzania	PhD student	Mutation induction of cooking banana for resistance to Fusarium wilt	4 months
Ms Susu ALKIERS	Austria	I	Plant breeding and genetics	8 months
Mr Phillipe NIKIEMA	Burkina Faso	F	Screening protocols for resistance to the parasitic weed *Striga*	4 months
Mr Michael HALL	USA	I	Data science	3 months

Crop Irradiation Services

In 2021, the PBGL irradiated 297 accessions/varieties for 25 MSs. Most requests were received in the context of Coordinated Research Projects (CRP) or Technical Cooperation Projects (TCP) with the remaining from stakeholder institutions from MSs, as summarized in the table below. The total number of irradiation requests now stands at 1683.

Request	Country	CRP/TCP	Species
1647	Namibia	TC	Groundnut
1648	Slovakia		Wheat
1649	Central African Republic	TC	Maize

Request	Country	CRP/TCP	Species
1650	Germany		Ornamental
1651	Hungary		Ornamental
1652	Serbia	TC	Soybean
1653	Kyrgyzstan	TC	Barley, wheat
1654	Poland		*Brassica napus*
1655	Croatia	TC	*Phaseolus vulgaris*
1656	Nigeria		Yam
1657	Greece	TC	Chickpea, sesame
1658	Germany		Ornamental
1659	Bosnia and Herzegovina	TC	*Phaseolus vulgaris*
1660	Burkina Faso	TC	*Digitaria exilis*
1661	Honduras	TC	Coffee
1662	The Netherlands		Ornamental
1663	Germany		Ornamental
1664	Poland	TC	Buckwheat
1665	Poland	TC	Barley
1666	Germany		Ornamental
1667	PBGL		Sorghum
1668	Tanzania	TC	Rice
1669	UK		*Euphorbia peplus*
1670	Namibia	TC	Groundnut, maize, bambara groundnut
1671	Eritrea	TC	Maize
1672	North Macedonia	TC	Common bean
1673	Iraq	TC	Peanut, mungbean
1674	Kenya	CRP	Sorghum
1675	Nigeria		*Vigna vexillata*
1676	Namibia	TC	Pearl millet
1677	Slovakia	TC	Wheat
1678	Croatia	TC	Wheat
1679	UK		*Papaver somniferum*
1680	Bulgaria	TC	triticale
1681	Greece	TC	*Pisum sativum*
1682	Zimbabwe	TC	maize
1683	Tanzania		banana

PUBLICATIONS and INFORMATION DISSEMINATION

AMRI-TILIOUINE, W., JANKOWICZ-CIESLAK, J., TILL, B.J., LAOUAR, M. Generation of Mutant Plants by Gamma Ray Exposure and Development of Low-cost TILLING Population in Chickpea (*Cicer arietinum* L.). In: Plant Gametogenesis: Methods and Protocols. Lambing, C, (eds.) Humana Press. (*in press*).

CÉSPEDES, R., ARRIETA, N., BARQUERO, M., ABDELNOUR, A., NIELEN, S., INGELBRECHT, I. (2021) Determination of Radiosensitivity of *Coffea arabica* var. Venecia Seeds to Gamma-ray Irradiation. In: Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change. S. Sivasankar *et al.* (eds.), CABI.

INGELBRECHT, I., AKGUN, E., MATIJEVIC, M., HOFINGER, B, JARC, L., JANKOWICZ-CIESLAK, J., ALI, A., JANKULOSKI, L., GRAUSGRUBER H., GHANIM, A.M.A. (2021). A Low-Cost Genotyping Protocol and Kit for Marker-Assisted Selection of Orange Lemma *(rob1.a)*, a Feed Quality Trait in Barley *(Hordeum vulgare* L.). Introductory guide and laboratory protocols. nafa-pbg-manual-diagnostic-marker-assay-ol-barleymay2021.pdf (iaea.org)

JANKOWICZ-CIESLAK, J., GOESSNITZER, F., DATTA, S., VILJOEN, A., INGELBRECHT, I., TILL, B.J. (2021) Induced Mutations for Generating Bananas Resistant to Fusarium Wilt Tropical Race 4. In: Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change. S. Sivasankar *et al.* (eds.), CABI.

JANKOWICZ-CIESLAK, J., GOESSNITZER, F., TILL, B.J., INGELBRECHT, I. Physical Mutagenesis and Population Development in Musa spp. In: Efficient Screening Techniques to Identify Mutants with TR4 Resistance in Banana. Springer Nature. (*in press*).

JANKOWICZ-CIESLAK, J., INGELBRECHT, I., TILL, B.J. Mutation Detection Using Low Coverage Copy Number Variation. In: Efficient Screening Techniques to Identify Mutants with TR 4 Resistance in Banana. Springer Nature. (*in press*).

JANKOWICZ-CIESLAK, J., WARTHMANN, N., MORALES ZAMBRANA, A.E., VILJOEN, A., INGELBRECHT, I. Induced Mutagenesis of Banana for Resistance to Fusarium Wilt Tropical Race 4. Plant & Animal Genome Conference XXIX, 08-12 January 2022, San Diego, poster Nr PO0173.

RABEFIRAISANA, H.J., GHANIM, A.M.A., ANDRIANJAKA, A., RASOAMAMPIONONA, B., JANKULOSKI, L., RAZAFINDRASOA, M.A., RAVELONJANAHARY, N.H., RAKOTOARISOA, N.V. (2021) Impact of Mulch-Based Cropping Systems Using Green Mulch and Residues on the Performance of Advanced Mutants Lines of Maize (Zea mays (L.)) Under Infested Field with the Parasitic Weed Striga asiatica (L.) Kuntze in Madagascar. In: Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change. S. Sivasankar et al. (eds.), CABI.

SIVASANKAR, S., ELLIS, T.H.N., JANKULOSKI, L., INGELBRECHT, I. (Eds). (2021). Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change**.** CABI.

TILL, B.J., HAWLICZEK, A., JANKOWICZ-CIEŚLAK, J., GUPTA, P., HOWARD-TILL, R., OSORIO, C.E., BOLIBOK-BRĄGOSZEWSKA, H. (2021) High-Throughput Discovery of Induced and Natural Mutations in Plants. Application Genetics of Plants – Challenges of the 21st Century, 22–24 September 2021 at the Warsaw University of Life Sciences (SGGW), Warsaw, Poland.
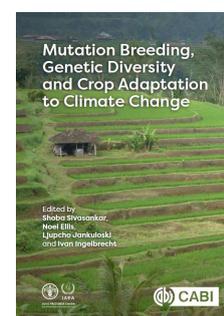
PBGL success stories

Combatting the Banana Wilt Pandemic with Nuclear Science

https://www.iaea.org/newscenter/news/combatting-the-banana-wilt-pandemic-with-nuclear-science

Climate Change and Coffee: Combatting Coffee Rust through Nuclear Techniques

https://www.iaea.org/newscenter/news/climate-change-and-coffee-combatting-coffee-rust-through-nuclear-techniques

THE SOIL AND WATER MANAGEMENT & CROP NUTRITION LABORATORY

EXECUTIVE SUMMARY

The Soil and Water Management & Crop Nutrition Laboratory (SWMCNL) of the Joint FAO/IAEA Centre 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 environmental pollution and nuclear emergencies affecting food and agriculture, as well in remediating their impact on soil and agricultural water resources.

In 2021, the SWMCNL conducted a wide range of activities: (i) it developed robust and affordable isotope, nuclear and related conventional techniques for climate-smart agriculture and it initiated research and development (R&D) on environmental pollution due to anti-microbial agents and micro-plastics; (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 develop climate-smart soil and water management practices and improve remediation of radioactive contamination in agriculture; (iv) carried out isotope analyses for R&D; and (v) provided quality assurance services to Member States.

Even though the year 2021 has been a year full of challenges, due to the COVID-19 pandemic, many R&D activities were implemented at the SWMCNL, including novel applications of isotope and nuclear techniques for climate-smart land and water management and remediation of radioactive contamination in agriculture. A new field of R&D was opened to develop stable isotope techniques to follow the fate of environmental pollutants, such as anti-microbial agents and micro-plastics. New tools for field scale soil moisture monitoring with Gamma Soil Moisture Sensors were tested. Important progress was also made in the development of protocols on the influence of nitrogen fertilization and clay mineral amendments on caesium dynamics in soils. All these activities are essential in supporting the implementation of the six Coordinated Research Projects (CRP) of the SWMCN Subprogramme, one of which is coordinated by the SWMCNL, i.e. CRP D1.50.19 on *'Monitoring and predicting radionuclide uptake and dynamics for optimizing remediation of radioactive contamination in agriculture'*.

A major component of the work of the SWMCNL is its significant contribution to training and capacity building in Member States. In 2021, the SWMCNL focussed on capacity building through the training and guidance of four PhD, three MSc students (through IAEA internships), two interns from seven countries in the use of nuclear and isotope techniques for climate-smart agriculture and nuclear emergency response.

R&D information was further communicated to Member States through 19 publications, including guidelines, protocols, conference papers and publications in international peer-reviewed journals, and one special issue on 'Sampling, analysis and modelling technologies for large-scale nuclear emergencies affecting food and agriculture' in the Journal of Environmental Radioactivity.

The SWMCNL analysed a total of 4659 samples for stable isotopes. 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$_2$O measurements using the laboratory-based laser isotope analysers.

STAFF

Name	Title	
Dercon, Gerd	Laboratory Head	94
Meniailo, Oleg	Soil Scientist	
Said Ahmed, Hami	Soil Scientist	
Heiling, Maria	Senior Laboratory Technician	
Resch, Christian	Senior Laboratory Technician	
Weltin, Georg	Senior Laboratory Technician	
Pucher, Reinhard	Laboratory Technician	
Toloza, Arsenio	Laboratory Technician	
Jagoditsch, Norbert	Technical Attendant	
Ajvazi, Ksenija	Team Assistant	
Faustmann, Aminata	Team Assistant	
Diels, Jan	Consultant	
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Mirkhani, Rayehe	Consultant	
Nakamya, Janice	Consultant	
Van Laere, Jonas	Consultant	
Vantyghem, Mathilde	Consultant	
Asanza, Megan	Intern	
Beelen, Eline	Intern	
Hategekimana, Innocent	Intern	
Martinez Maya, Maria Alejandra	Intern	
Rohling, Mike	Intern	

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 environmental pollution and 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 gases 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.

Near-real-time Web GIS tool for Climate Smart Water Management (CSWM) by combining Cosmic-Ray Neutron Sensor data and remote sensing data (Sentinel 1 & 2 and MODIS)

Climate change and its impact on water resources is a major problem for agricultural production. To enhance agriculture production in a sustainable way, there is a need to develop climate-smart agricultural practices to improve water-use efficiency. For agricultural water management, crop evapotranspiration (ET) and soil moisture are the key indicators for crop water requirement. Many remote sensing-based methods have been developed to measure ET at a global scale using satellite and airborne imagery. For large scale soil moisture monitoring, nuclear technology, such as Cosmic-Ray Neutron Sensors (CRNS), bridges the critical gap between satellite and point-scale ground sensors. It can enable the calibration of satellites such as Sentinel-1 (S1) to improve soil moisture data estimated by remote sensing.

In 2020 the SWMCN laboratory started to work on the development of a near-real-time Google Earth Engine Web GIS tool for estimating soil moisture by combining CRNS data and remote sensing data (Sentinel 1 & 2 and MODIS), using highly spatial resolution (10 m) and highly temporal resolution (5 days) data. This year the team included an additional model and drought monitoring:

- Evapotranspiration by combining the WAPOR (Water Productivity Open access of Remotely sensed data) database and temperature data from MODIS.

- Drought water stress by using the Normalized Difference Drought Index (NDDI) which is obtained from a combination of Normalized Difference Vegetation Index (NDVI) and Normalized difference Water Index (NDWI), which are sensitive to vegetation and water content of vegetation, respectively.

This was applied to produce a highly spatial resolution (10 m) soil moisture map for date palm fields in Kuwait, linked to one of the IAEA Technical Cooperation projects supported by the SWMCNL.

For the first time, in a semi-arid region, important parameters such as soil moisture, crop water requirement, crop greenness and drought status were made accessible and combined for agricultural research organizations through a Web GIS platform combining nuclear technology and satellite imagery. Farmers and scientists may freely use this platform to gather important information to develop climate smart agriculture (Figure 1).
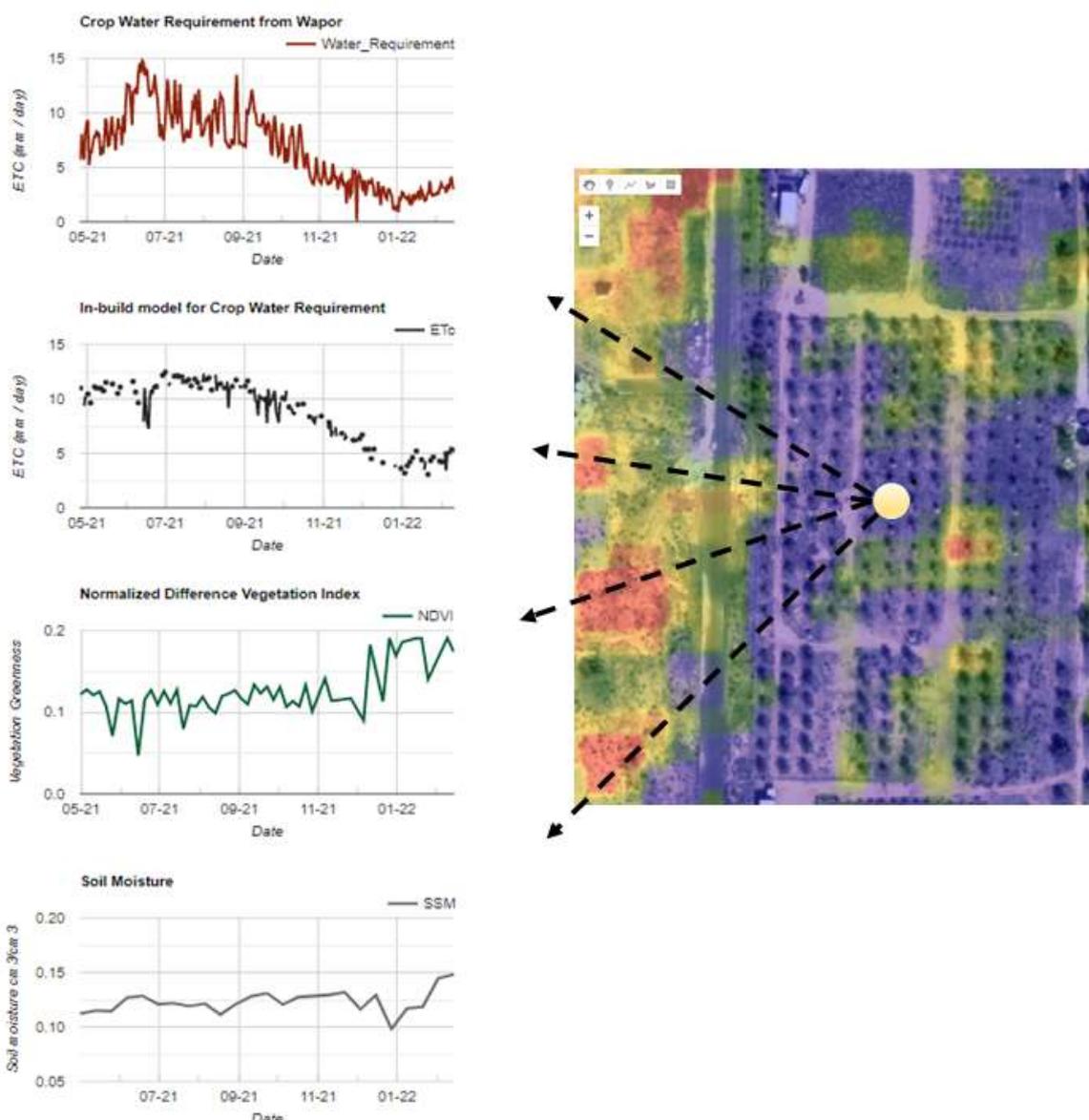
Figure 1. *Screenshot of the Web GIS platform for a date palm plantation in Kuwait (data presented from May 2021 until January 2022).*

Field scale soil moisture monitoring with Gamma Soil Moisture Sensors

More recently a new nuclear technology based on the use of Gamma Ray Sensors (GRS) (Figure 2), emerged as non-invasive method for soil moisture monitoring at a smaller field level. The GRS approach for estimating soil moisture is based on the link between soil moisture and gamma-ray

intensity of the [40]K radionuclide present in the soil matrix. With an estimated footprint of about 0.2 to 1 ha depending on the height of the sensor, the GRS can be suitable for soil moisture monitoring in small scale fields and irrigation schemes. This technology is relatively new, and few studies have attempted to estimate soil moisture using gamma-ray intensity monitoring.

Therefore, the SWMCN Laboratory started to work on evaluating the ability of the GRS for field soil moisture monitoring, at its experimental fields in Austria. The first results showed that the GRS retrieves the soil moisture variation for example after a rainfall event (Figure 2). This suggests that GRS is a promising tool for soil moisture monitoring in small scale agriculture which is largely predominant in many Member States. The SWMCN will continue to develop methodologies on the use of the GRS for water-saving agriculture.
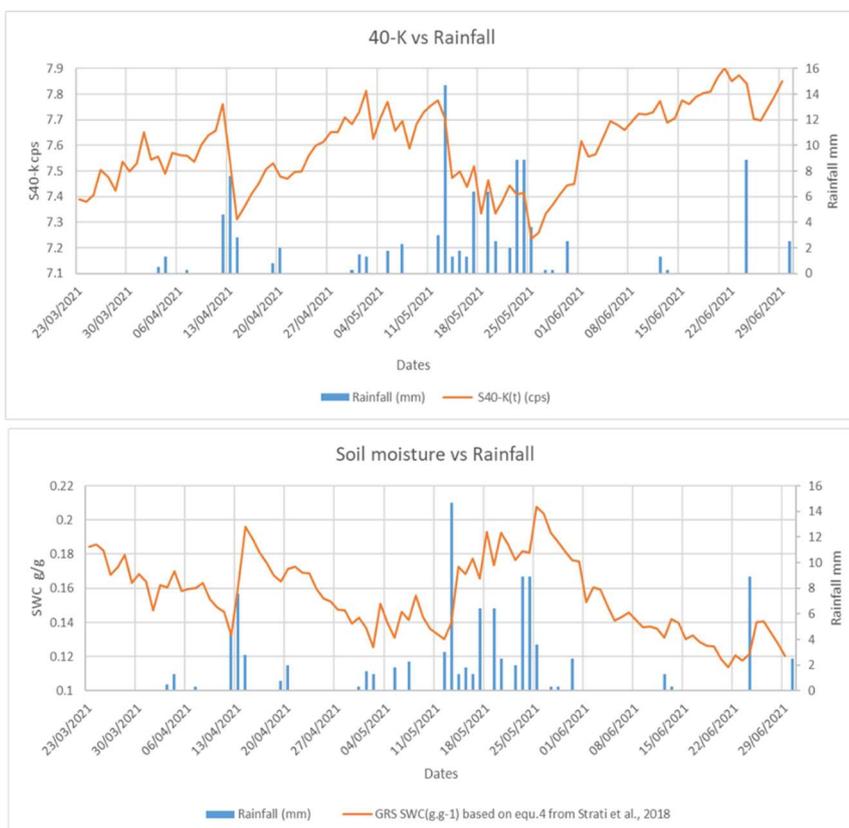
Figure 2. *Gamma Ray Sensor (GRS) data ([40]K) and derived soil moisture information in relation to rainfall events, in the SWMCNL experimental fields of Rutzendorf (Austria).*

Influence of nitrogen process inhibitors on ammonia volatilization

The release of large amounts of ammonia (NH_3) after the application of urea is a serious problem, which not only reduces economic returns to farmers, but also has a negative impact on the environment. Mitigating NH_3 losses from nitrogen (N) fertilizer application thus provides a double benefit for farmers and the environment.

In this study, our objective was to understand the effect of N process inhibitors on daily and cumulative NH_3 volatilization. Nitrogen inhibitors are products that temporarily retard conversion of fertilizers to the forms that can be lost through different pathways and can improve N use efficiency (NUE) and consequently enhance crop yield and reduce environmental emissions. There are two main types of inhibitors that can be added to N fertilizers:

- Urease inhibitors (UI) slow down the hydrolysis of urea
- Nitrification inhibitors (NI) inhibit the biological oxidation of ammonium to nitrate

A field experiment was established at the SWMCNLin Seibersdorf, to determine the effect of different N fertilizers coated with N process inhibitors on maize yield and NH_3 volatilization. The field site is characterised as a moderately shallow chernozem soil with significant gravel content. A randomized complete block design with four replications was used in this study. Treatments were: T_1 (control treatment - without N fertilizer), T_2 (Urea only), T_3 (Urea + UI), T_4 (Urea + UI + NI-1), and T_5 (NPK + NI-2). NI-1 referred to MPA: N-[3(5)-methyl-1H-pyrazol-1-yl) methyl] acetamide while NI-2 is DMPP: 3,4-dimethylpyrazole phosphate. Urea was applied through two equal split applications in the T_2 treatment (at 20 days after planting (DAP) and 34 DAP). In T_3, T_4, and T_5 treatments, N fertilizers were applied only once (at 20 DAP). All treatments received 120 kg of N ha^{-1}, except of treatment 1, 60 kg ha^{-1} of P_2O_5 and 146 kg ha^{-1} of K_2O. Supplemental irrigation was only applied in the early stages of growth, to ensure that the crop could establish. Ammonia volatilization was measured with semi-static chambers. Measurements were taken every two days during the first month, then every three days for the second month.

The first results were reported in the previous annual lab report, showing a clear impact of the N process inhibitors on maize yield. In this report, we emphasize the temporal patterns of ammonia volatilization from nitrogen fertilizers coated with inhibitors.

The results showed, an emission factor of 22%, 18%, 13%, and 7% of the applied nitrogen for T2, T3, T4, and T5 treatments, respectively. Figure 3 shows that all treatments resulted in a sharp increase in NH_3 emissions after addition of fertilizers. In T_2 (U), the emissions after the first and second splits showed similar time course and reached a peak on day 4 after the application of the fertilizers. The pattern of daily NH_3 losses from urea applied alone in the first and second splits were similar, confirming that the majority of NH_3 losses occurred during the first few days following urea application. By contrast, the emissions in treatments T_3 (U+UI) and T_4 (U+UI+NI-1) were lower and the increase persisted longer than in the urea alone treatment (T_2). The addition of UI to urea in T_3 and T_4 treatments reduced the peaks of the NH_3 loss, delaying them until day 8. As T_5 treatment (PK+NI-2) is composed of nitrate and ammonium, the volatilization losses of NH_3 from this treatment should theoretically be lower than other urea treatments. This was confirmed by our results.

In urea alone treatment (T_2), measurements of the daily NH_3 emission showed that about 80% of NH_3 losses occurred within seven days after each split application and dropped quickly. For U+UI (T_3) and U+UI+NI-1 (T_4) treatments, the majority of NH_3 losses (between 76-77%) happened within two weeks after adding nitrogen fertilizer. In NPK+NI-2 treatment (T_5), using composed of nitrate and ammonium, about 63% of NH_3 losses occurred within the first week.
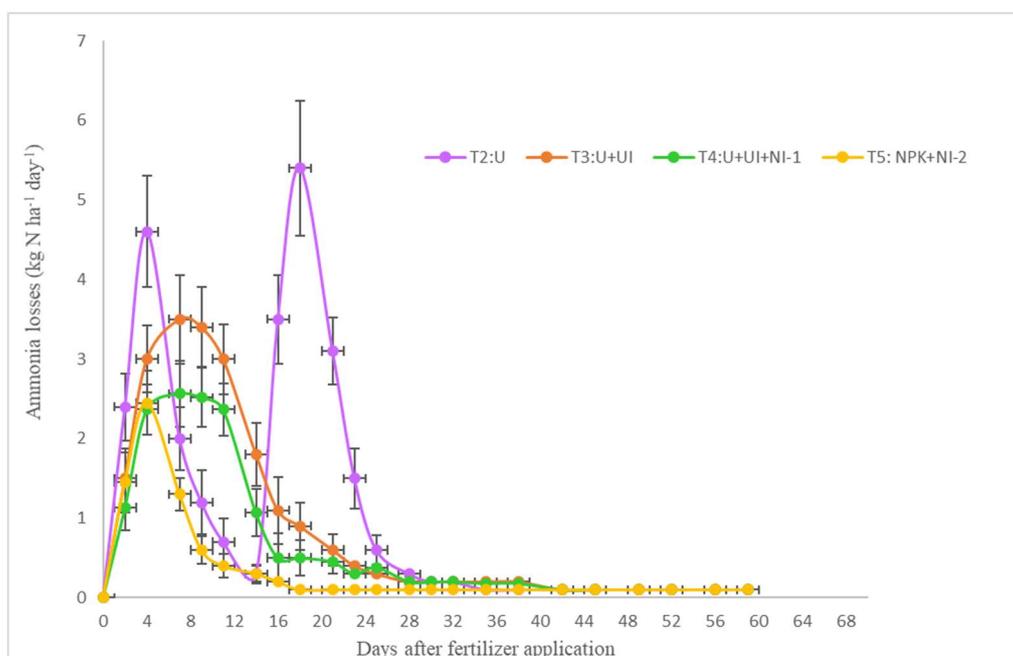
Figure 3. *Daily NH₃ emissions of different fertilization treatments. Each value represents a mean of four replicates with standard deviation shown by vertical bars.*

Understanding the interaction between maize water use efficiency and nutrient uptake in irrigated cropping systems, a basis for predicting and improving Zambia's productivity in a changing climate

The agriculture sector is a key contributor to the Zambian economy and essential to ensure food security in the country. However, climate change has a negative impact on Zambian agricultural production. In line with its Vision 2030 to have an efficient, competitive, sustainable, and export-led agriculture sector, Zambia is making efforts to improve irrigated agriculture through investments in various irrigation projects. Irrigated agriculture has the potential to secure farmers income but at the same time may impact the environment if resources are not properly managed. This study aims to improve irrigation management by optimizing water and nutrient (N) use efficiency for maximum crop water productivity at field levels. This will be used to predict and compare the effects of climate change on different water and nutrient application levels. To achieve this goal, the research will use and adapt nuclear and isotope techniques for the Zambian agro-ecological conditions. Drip irrigation will be the targeted system. The experimental design is as follows: (a)Water application levels in maize crop (deficit [50% and 75%] versus optimal irrigation; and (b) N levels in maize crop (140 kg N/ha, 112 kg N/ha and 84 kg N/ha, optimal and widely practiced being 112 kg N/ha).

Maize was grown as a sole crop, in rotation with a legume under drip irrigation, over the 2021 dry season, April-October, with three (3) levels of water application; (i) optimal (required amount as per crop water requirement), (ii) deficit (50%) (iii) deficit (75% of optimal) and three (3) levels of N. The rates of water (deficit and optimal) applied were determined by calculating the evapotranspiration using local climate data, FAO's New LoClim, CROPWAT software and root-zone soil water balance approach. Data collected include soil moisture monitoring with a neutron probe and punched leaves of plants fertilized with ¹⁵N enriched fertilizer. Grains and stalk samples of enriched plants were also analysed.

Preliminary results show a significant difference in the nitrogen treatments and the age of the leaves. This result will be used to compare with the bulk samples' N levels to recommend a less destructive method of ¹⁵N tracing analysis. The yield indicates that water levels of 75% of optimum

gave a comparatively good yield while 112 kg/ha of N can be recommended for this project area. Therefore, in this project area, water levels can be reduced to 75% (translating to a water saving of 1,073 m³/ha), and fertilizer be maintained at the widely used level of 112 kg/ha and the yield would not be affected (figure 4).
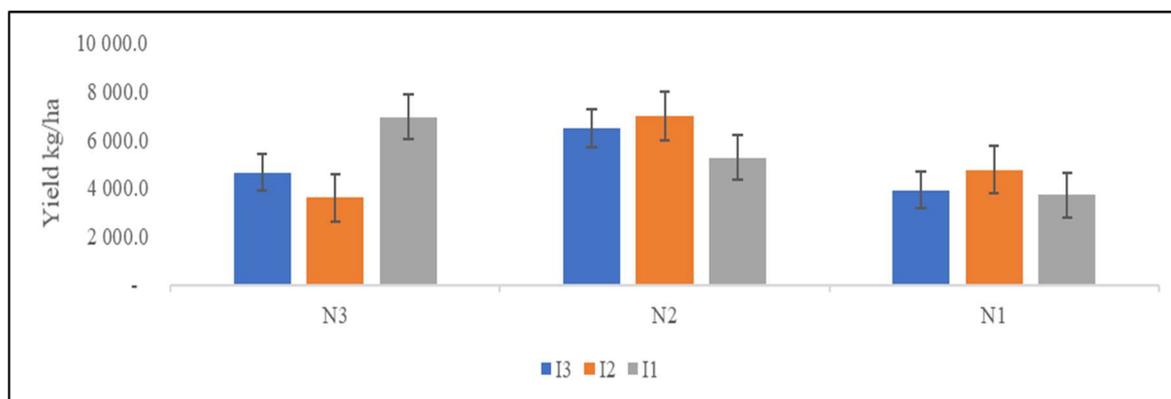
Figure 4. *Maize yield in the study site, where N3, N2, N1 represents 140, 112 and 84 Kg N/ha, and I3, I2 and I1 refers to an optimum, 75%, and 50% water level respectively.*

This project has been supported by the International Centre for Theoretical Physics (ICTP)/IAEA Sandwich Training Educational Programme (STEP). Through this fellowship, the fellow Mumba Mwape can join the SWMCNL team three times over the next three years. You can find more information about ICTP/IAEA STEP opportunities on the following website: ICTP - ICTP/IAEA Sandwich Training Educational Programme.

Assessing water use efficiency and drought stress in cassava: Establishing sampling strategies for stable carbon and oxygen isotope techniques

Climate change is affecting weather patterns all over the world. One of the effects of climate change in Central Africa is expected to be an increase in dry spells. These dry spells will decrease the production of staple crops such as cassava[4], affecting millions of people in that region. The Consortium for Improving Agriculture-based Livelihoods in Central Africa (CIALCA, www.cialca.org) is therefore aiming to increase cassava yields with the predicted climate change in mind. Several agronomical practices are being investigated to make cassava cropping systems more resilient against climate change. Variety selection and fertilizer application are the two main components in the battle against climate change which are being investigated in the SWMCNLunder the CIALCA project.

In 2021, a greenhouse experiment was conducted in the SWMCN greenhouses. This experiment was mainly focusing on the influence of variety selection and potassium (K) application on drought tolerance of cassava. Shortly, a one-month period of drought was imposed to one improved (Narocass1) and one local variety (Gacyaricyari) at five months after planting to half of the plants (W+ and W- treatment). Of these plants, half received optimal K nutrient solution (K+ treatment), while the other half received suboptimal quantities of K (K- treatment). Plants were harvested six months after planting. At harvest both the youngest fully expanded leaf and the leaf that was the youngest fully developed leaf right before the onset of drought were analysed for stable isotopes.

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[4] Daryanto S, Wang L, Jacinthe P-A (2016) Drought effects on root and tuber production: A meta-analysis. Agric Water Manag 176:122–131. https://www.sciencedirect.com/science/article/pii/S0378377416301810

Data on morphology, physiology as well as isotopic information were collected for all plants and effects of the treatments were assessed. Based on analysis of variance (ANOVA) results, we can see that Narocass1, which is the improved variety, has a significantly better transpiration efficiency (*p<0.001*) compared to the local variety (Figure 5). With the same amount of water transpired, 85% more root biomass is produced in the improved variety. When we zoom in to the improved variety, we can also see that plants receiving the K+ solution, produced 79% more biomass per litre transpired water (*p<0.05*). This same effect of K could however not be seen in the local variety. Overall, there was no significant effect of the induced drought on the transpiration efficiency in both varieties.

Isotopic composition of leaves was also measured to assess its relationship to transpiration efficiency (linked to root biomass or to total biomass). Bulk leaf samples, extracted cellulose and extracted sugars were analysed for C-13. Correlation between the δ^{13}C and transpiration efficiencies can be found in Table 1.

We can extract from Table 1 that the most significant correlations are found in the tagged leaf and not the youngest fully expanded leaf, which is a commonly used leaf for plant diagnostics. Overall, the bulk δ^{13}C of the tagged leaf gave the strongest correlations. Plants with a higher transpiration efficiency, showed lower δ^{13}C values for all components that had a significant correlation. Zooming in to each variety, we can see that Gacyaricyari has mostly stronger correlations than the improved variety.
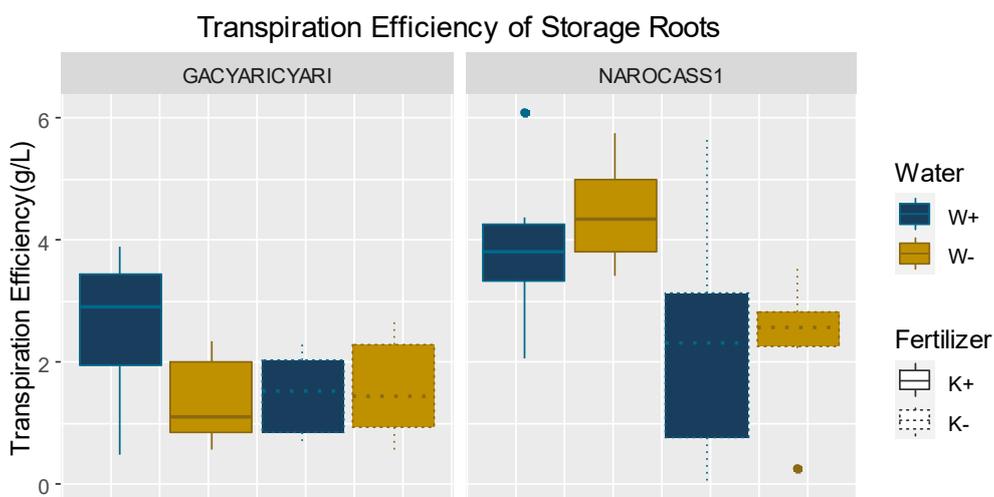
Figure 5. *Transpiration efficiency of the storage roots (grams of roots produced per liter of water). Blue color represents W+ treatment at 90% of pot capacity, brown color represents W- treatment at 50% of pot capacity. Full box edges are K+, while dotted edges are K- treatment. Number of replicates per group is 6.*

Table 1. *Pearson correlation coefficients between δ^{13}C of bulk, cellulose, and sugars in either youngest fully expanded leaf (YFEL) or the tagged leaf and transpiration efficiency (TE) of the total biomass or root biomass for two contrasting varieties. The significance of the correlation is indicated with stars (*<0.05, **<0.01, ***<0.001)*

	Gacyaricyari		Narocass1		Both varieties	
	TE_{total biomass}	TE_{roots}	TE_{total biomass}	TE_{roots}	TE_{total biomass}	TE_{roots}

YFEL	$\delta^{13}C_{BULK}$	-0,47*	-0,49*	-0,13	-0,31	-0,28	-0,42**
	$\delta^{13}C_{CELLULOSE}$	-0,47*	-0,52*	0,10	-0,09	-0,14	-0,31*
	$\delta^{13}C_{SUGAR}$	-0,35	-0,26	-0,45*	-0,44*	-0,24	-0,13
Tagged leaf	$\delta^{13}C_{BULK}$	-0,68***	-0,77***	-0,56**	-0,59**	-0,59***	-0,64***
	$\delta^{13}C_{CELLULOSE}$	-0,58**	-0,72***	-0,09	-0,24	-0,40**	-0,57***
	$\delta^{13}C_{SUGAR}$	-0,44*	-0,42*	-0,59**	-0,51*	-0,39**	-0,27

Unraveling carbon and water dynamics in banana mats through a ^{13}C labelling experiment

Following earlier steps in the PUI (Peaceful Uses Initiative) project on *Enhancing climate change adaptation and disease resilience in banana-coffee cropping systems in East Africa*, funded by Belgium, a more in-depth experiment was performed in the SWMCNL greenhouses to investigate the translocation of carbon-assimilates in banana mats under different watering treatments. Earlier findings showed the potential of the δ^{13}C signature as a short-term indicator for drought stress. In this experiment, enriched $^{13}CO_2$ label was used as a tracer to quantify carbon (C) fluxes from source to sink within the plant. The major emphases were the measurement of the carbon transfer from mother to daughter plant and determination of the potential influence of drought stress on this process.

Twenty-four banana plants were subjected to two different water treatments (optimal, 100% field capacity and suboptimal, 50% field capacity). Half the plants consisted of a sole mother plant and half of the plants had a daughter plant attached to the mother plant. All plants were labeled with a $^{13}CO_2$ pulse in the SWMCNL growth chamber, whereby the soil and daughter plants were covered to avoid direct uptake of the ^{13}C enriched CO_2 *(Figure 6)*. This way, only mother plants would uptake the labeling, allowing us to trace the path of photo-assimilates. After labeling, samples were taken from different plant parts at specified time moments *(Figure 1)* for δ^{13}C analysis. Extractions were done on the samples to isolate mobile (sugars) and immobile (starch and cellulose) carbon.

Figure 6. *Preparation of plants before entering the growth chamber for the ^{13}C pulse labelling where the daughter plant and soil are packed in a vacuum bag in polyethylene and the opening is sealed with tyrosine clay and taking a phloem sample from a petiole of the mother plant for water soluble organic matter extraction.*

Preliminary results indicate that there is indeed a carbon flux from mother to daughter plant (*Figure 7*). After labeling, the δ^{13}C value of daughter plant leaves increases, which implies ^{13}C is being received from the mother plant. The maximum δ^{13}C values are visible at 72 hours after labeling, although the delta value of the youngest leaf (leaf 0, which is not completely developed) continues to increase until 120 hours after labeling.

Further analysis will allow us to quantify this flux, as well as to determine the effect drought stress has on the process. By understanding these effects, we can formulate better recommendations for farmers with regards to plant management under drought stress. Moreover, this experiment will contribute to a better understanding of carbon dynamics in banana, a historically understudied and poorly understood crop.
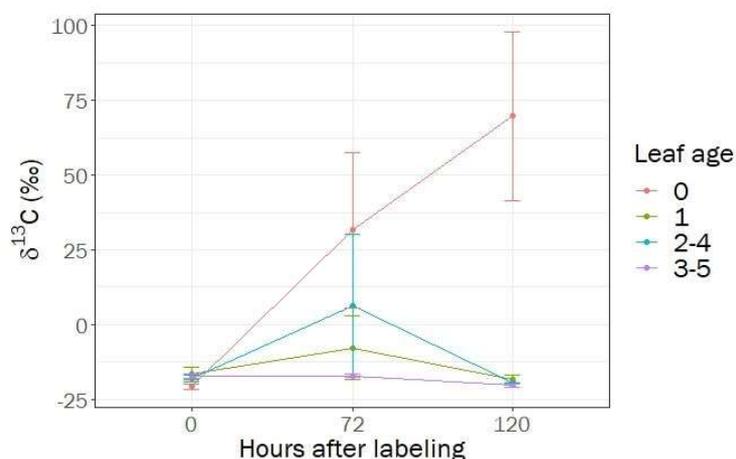
Figure 7. *Stable carbon isotope values (δ^{13}C) of different leaves of banana daughter plants after pulse labeling with ^{13}C. The leaf age refers to the order of opening whereby leaf 1 is the most recently fully opened leaf, leaf 0 is a partially opened leaf and leaf 2-5 are the respective older leaves that are fully opened. Error bars indicate standard error (n = 2-10).*

AquaCrop cassava assessment

To enhance food security and income of cassava farmers through increased productivity, resource use efficiencies and climate resilience, the SWMCN Laboratory and Section launched the initiative to develop a cassava crop-file for the AquaCrop model. This initiative was supported by the Regional TC project RAF5081 on Enhancing Productivity and Climate Resilience in Cassava-Based Systems through improved Nutrient, water and Soil Management, and the Consortium for Improving Agriculture-based Livelihoods in Central Africa (CIALCA, www.cialca.org).

FAO's field-crop-water-productivity model, AquaCrop, was calibrated and validated for the case of cassava. The model simulates attainable crop biomass in response to soil moisture variations. Although based on basic and complex biophysical processes, the model uses a relatively small number of parameters to be adjusted according to the case and crop. AquaCrop balances accuracy, simplicity and robustness, and it is particularly well suited to conditions in which water is a key limiting factor in crop production.

Several climate, soil, canopy cover and biomass datasets were: i) retrieved from the *Decision Support System for Agrotechnology Transfer (DSSAT)* model (a software application program that comprises crop simulation models for over 42 crops; as of Version 4.7.5) for the CIAT site in Colombia, ii) shared by the *International Fertilizer Development C (IFDC)* in Togo and iii) the *African Cassava Agronomy Initiative (ACAI)* project of *International Centre for Tropical Agriculture International Institute of Tropical Agriculture (IITA)* in Nigeria; covering several varieties, years and regions.

A single crop file was created for the ensemble of South American and West-African experiments. Simulation performance was assessed by comparing observed and simulated dry total biomass. Results gave an overall R^2 of 0,90; RMSE of 2,0 t/ha and rRMSE of 3,5%. Final yield is not yet implicated, since harvest indices were too cultivar specific and ranged from 45 to 60%; and not enough final yield observations were available for each variety. An example for the MPtr-26 variety in Palmira (Columbia) is given in Figure 8.

The resulting cassava crop-file will be added in the upcoming updated AquaCrop version 7. A detailed article on the calibration and validation procedure and results is published open-access in Agricultural Water Management (Calibration and validation of the FAO AquaCrop water productivity model for cassava (Manihot esculenta Crantz) - ScienceDirect).

(a)

(b)

(c)
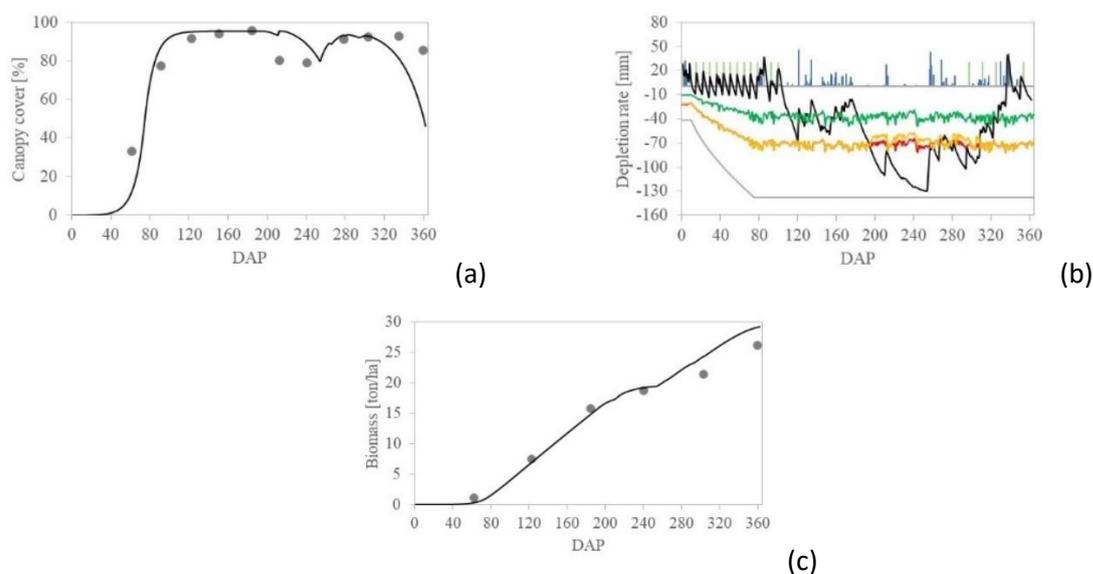
Figure 8. *AquaCrop cassava simulation for the MPtr-26 variety for Palmira (Columbia, 1980); a) simulated (line) vs observed canopy cover (dots); b) root zone water depletion (black line), depletion at saturation and permanent wilting point (grey dashed lines), and different water stress thresholds (green: canopy expansion; red: stomatal closure; orange: early senescence); c) cumulated biomass (line) vs observed biomass (grey dots).*

Environmental pollution

SWMCNL has opened a new field of research in anti-microbial resistance (AMR) and microplastics

An acute soil pollution problem is the growing antimicrobial resistance (AMR). AMR is defined as the ability of microorganisms to withstand the effects of antimicrobials. Antimicrobials (AM) are used to treat bacterial and other microbial infections in both humans and animals and are also used as a growth promoter in animals. Agriculture can thus be a source for AMR, which is currently one of the most serious health problems affecting global health with about seven hundred thousand deaths per year. The recently initiated FAO/IAEA Coordinated Research Project (CRP D1.50.22) on *'Isotopic Techniques to Assess the Fate of Antimicrobials and Implications for Antimicrobial Resistance in Agricultural Systems'* targets sulfamethoxazole (SMX) as a model AM and will determine the fate of this AM in agricultural systems under field conditions. The SWMCN Laboratory will support the ongoing CRP by conducting several incubation experiments with ^{13}C-labeled SMX. This would provide us with some insights on SMX turnover in soils, which might help designing a strategy for studying the fate of SMX under field conditions. While a lot of previous research on SMX degradation was carried

out in water tanks, less knowledge is available on SMX mineralization in soils. We would study also the temperature and soil moisture sensitivity of SMX mineralization in soils under the influence of N and P fertilizers. Also, there is a gap in knowledge how SMX affects soil microbial community, activity of C cycling in soils. We plan to conduct an experiment with ^{13}C-glucse application together with unlabeled SMX, clarifying the SMX effects on soil C mineralization, microbial priming of soil organic matter, and glucose mineralization.

Another significant source for pollution of agricultural soils are microplastics (MP). The FAO/IAEA SWMCN Subprogramme aims to contribute to this field by launching a new IAEA funded Coordinated Research Project (CRP) in 2022. A major gap in the field of microplastics is the lack of understanding of its behaviour in soil and its biogeochemical impact. Stable isotopes can be successfully used to study the degradation or mineralization of MP. While chemically produced plastics are inert in soils and not mineralizing, biodegradable plastics can substantially reduce the problem of soil contamination. Isotopes can be used to track the products of biodegradable MP decomposition, to determine the rate of their degradation under different soil conditions (temperature, moisture, fertilizer application), revealing the effects of MP's presence on soil C turnover and greenhouse gas fluxes.

In 2021, several pieces of equipment were purchased and installed at the SWMCN Laboratory. This includes a) Gas Chromatography-Isotope Ratio Mass Spectrometry (GC-IRMS) for compound-specific multi-element stable isotope fingerprinting, b) CRDS analyzer (Picarro 2201-i), which precisely and continuously measures δ^{13}C in carbon dioxide (CO_2) and in methane (CH_4), (c) Peltier-technology incubator Memmert for incubation of soil samples amended with microplastics at different temperature regimes. We intend to assemble an automated incubation system, where soil samples will be incubated for days or weeks, unattained by operator, with constantly monitoring the CO_2 and CH_4 fluxes and their isotopic composition. With this new automated system, we hope to obtain new data on temperature and moisture sensitivity of microplastics or antibiotics decomposition. With GC-IRMS we are determining ^{13}C incorporation into phospholipid-derived fatty acids (PLFAs), allowing estimation the proportion of microplastics-C antibiotics-C assimilated either by bacterial or by fungal biomass.

Altogether, the research experiments on isotopically labeled SMX and naturally labeled biodegradable microplastics would provide important new insights on environmental effects on the SMX and microplastics mineralization in agricultural soils and the effects of SMX and microplastics on soil microbial life and the processes of soil C cycle.

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. Further emphasis is now also put by the SWMCNL on the optimization of remediation of radioactive contamination in agriculture.

The influence of ammonium fertilization and clay mineral amendments on caesium dynamics in different soils

In the aftermath of a nuclear emergency, remediation of radioactive contamination is crucial to ensure food safety within affected regions. It is important to investigate soil properties and consider specific

agricultural practices in planning remediation. Radiocaesium is one of the key radionuclides that raises concern for food safety.

 The implementation of this research activity is under the Coordinated Research Project D1.50.19 launched in 2019 which aims to enhance readiness and capabilities to optimize remediation. It emphasizes on under-explored agro-ecological environments to design an optimal remedial model. The SWMCN Laboratory, in close collaboration with the National Agriculture and Food Research Organization (NARO-Japan) and BOKU (University of Natural Resources and Life Sciences Vienna, Austria), has been evaluating the influence of ammonium (NH_4^+) fertilization and clay amendment in soil-to-plant transfer of radiocaesium since the beginning of 2021.

Nitrogen (N) fertilizers are used widely around the world to improve crop yield. Many of which are applied as NH_4^+ since it is retained in soils due to its ability to occupy the negatively charged sites of organic matter and clay minerals. However, NH_4^+ and caesium (Cs^+) compete for a position in these negatively charged sites. The release of Cs^+ into soil solution, from which plant roots directly take up nutrients and pollutants, can be caused by NH_4^+ fertilization and consequently facilitate Cs^+ plant uptake.

 During the 2021 R&D at the SWMCNL, we focused on the behavior of NH_4^+ in connection with soil solution Cs^+ in Austrian, Belarusian, and three Japanese soils with strongly varying clay quantities and characteristics.

In this report, we highlight the results related to the effect of a 300 kg ha^{-1} NH_4^+-N fertilization and clay amendments on Cs^+ dynamics in the case two Japanese soils. This first soil is an allophanic Andisol, a typical Japanese soil with low NH_4^+ and Cs^+ selectivity formed on volcanic ash. The second soil is considered as a vermicultic Cambisol with high Cs^+ selectivity.

NH_4^+ - fertilization led to drastically raised NH_4^+ as well as Cs^+ concentrations in soil solution in the beginning of the observation period for the Andosol (Figure 9 and 10). Without clinoptilolite amendments, NH_4^+ concentrations reached 12.5 mmol L^{-1} while Cs^+ raised up to 107 nmol L^{-1} after one day. However, a strong NH_4^+ decrease could be observed between 8 and 22 days. This phenomenon can generally be explained by nitrification processes, which cause a decrease in NH_4^+ and a simultaneous increase in NO_3^-. This hypothesis was also supported by additional collected data for this experiment.

The application of 40t ha^{-1} clinoptilolite caused a significant ($\alpha = 0.001$) reduction of NH_4^+ as well as Cs^+ concentrations in soil solution after day 1 and 8, whereby effects on day 22 and 36 were small. Cs^+ was stronger adsorbed than NH_4^+ on the first day, as its concentration decreased almost 14-fold while NH_4^+ concentration decreased only threefold, supporting clinoptilolite´s high Cs^+ selectivity (Valcke et al., 1997). Previous results, which suggested an increase of NH_4^+ concentration after 22 days could be confirmed but occur on a low concentration level and did not affect Cs^+ concentrations in soil solution. As slow release of NH_4^+ after initial NH_4^+ fertilization can be beneficial for optimal plant growth, particularly under humid climate in which NO_3^- is easily lost from topsoil due to leaching, a synergy between remediation and agricultural practice can be assumed.

Results of the vermiculitic Cambisol differed significantly to that of the allophanic Andosol. Even though fertilisation and time drastically changed solution NH_4^+ (Figure 11), those did not affect Cs^+ in soil solution at all (Figure 12). Most likely, this is due to specific Cs^+ retention capacities of vermiculites. In contrast to Clinoptilolites, which exhibit indeed high Cs^+ selectivity, vermiculites have the additional capacity to hinder Cs^+ desorption which strongly favours remediation of radiocaesium contaminated soils (Ferreira et al., 2021).

Summarizing, clinoptilolite application can be a useful measure to decrease Cs^+ in soil solution and thereby the probability of radioactive contamination of crops. However, it needs to be considered that it also leads to an increase of negative charges in soils and can remove positively charged plant nutrients as NH_4^+ from soil solution. Furthermore, a decrease in K^+ in soil solution provokes less selective uptake mechanisms in plant roots and so enhances the risk of Cs^+ uptake, as could be seen in investigations of Dengra I Grau (carried out in 2020). In addition, the Cambisol proved to be resistant towards Cs^+ release after NH_4^+ fertilization, indicating the importance of vermiculites in the context of radiocesium remediation. A further step of this experiment will be the determination of K^+ in soil solution to better understand its interplay with NH_4^+ and Cs^+. Another focus will be on the differences among the investigated soils and the effects of different applied clay minerals and application rates. Future research should focus on actual plant uptake of Cs^+ and its modulation to calculate even better the risk of radiocaesium contaminated soils and therefore the risk for human health.
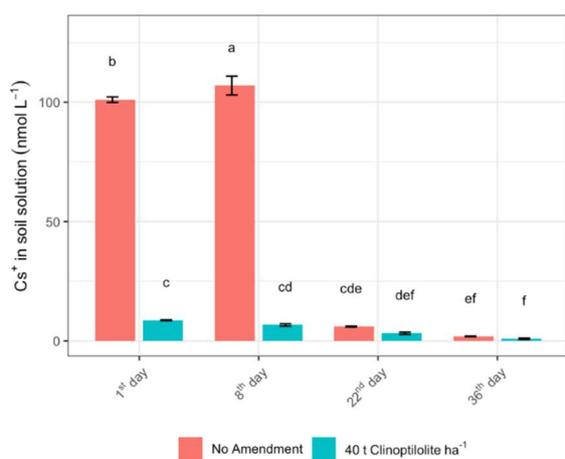
Figure 9. Cs^+ dynamics with 40t ha^{-1} Clinoptilolite and no amendments after NH_4^+ fertilisation. Different letters indicate significant differences between different days and treatments, with significance level of α = 0.05 (Tukey-Test).
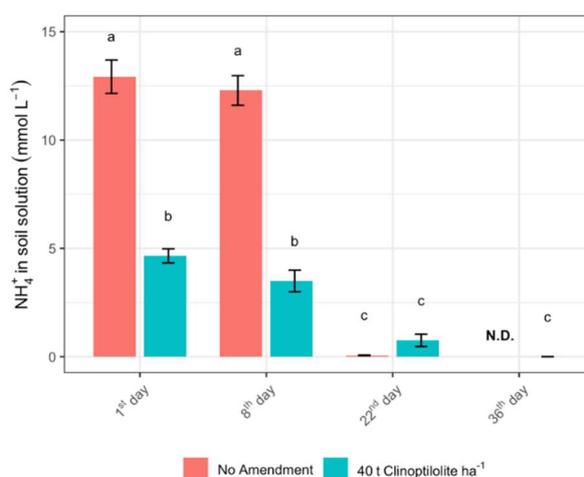
Figure 10. NH_4^+ dynamics with and without zeolite amendments after initial NH_4^+ fertilisation. Different letters indicate significant differences between different days and treatments, with significance level of α = 0.05 (Tukey-Test). N.D. indicates values below the detection limit.
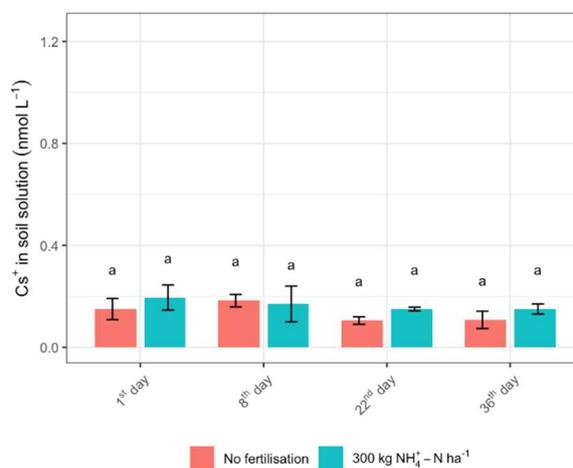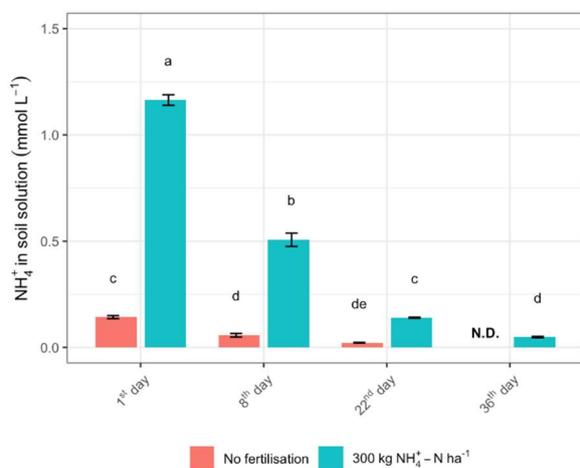
Figure 11. *NH₄⁺dynamics over time after NH₄⁺ fertilisation and no fertilisation. Different letters indicate significant differences between different days and treatments, with significance level of α = 0.05 (Tukey-Test). ND values were below detection level.*

Figure 12. *Cs⁺ dynamics over time after NH₄⁺ fertilisation and no fertilisation. Different letters indicate significant differences between different days and treatments, with significance level of α = 0.05 (Tukey-Test). ND values were below detection level.*

Prediction of Exchangeable Potassium in Soil through Mid-Infrared Spectroscopy and Deep Learning: from Prediction to Explainability

Within the context of nuclear emergency response, the high-throughput characterization of soil properties in general and exchangeable potassium in particular are of critical importance for remediating radioactive contamination in agriculture.

Indeed, in case of nuclear emergencies affecting food and agriculture, exchangeable potassium plays a major role. As potassium competes with radiocaesium in soil-to-plant transfer, it can help reduce the uptake of this major fallout radionuclide. The ability to characterize exchangeable potassium rapidly and repeatedly will inform and facilitate decision making, and significantly reduce the cost of analyses needed for remediation over the years.

Traditional methods for soil property characterisation through wet chemistry provide high accuracy and precision, but it is often cost prohibitive. As an alternative, Mid-Infrared Spectroscopy (MIRS) and Statistical Learning allow on the contrary the high-throughput characterization of soil physical, chemical and biological properties. However, models developed were often limited in scope as only small and region-specific MIR spectral libraries of soils were accessible. Moreover, under this data regime, models developed (e.g Partial Least Squares) failed to characterize exchangeable potassium to an acceptable level of accuracy.

The situation of data scarcity is, however, changing rapidly today with the availability of a large and growing high-quality library of MIR-scanned soil samples maintained by the National Soil Survey Center (NSSC) Kellogg Soil Survey Laboratory (KSSL) from the United States Department of Agriculture (USDA-NRCS). Through the Global Soil Laboratory Network (GLOSOLAN, https://www.fao.org/global-soil-partnership/glosolan) of the Food and Agriculture Organization (FAO), and initiatives such as Soil Spectroscopy for Global Good (https://soilspectroscopy.org), this database was made available. The unprecedented volume and diversity of data now available allows soil science researchers to increasingly shift their focus from traditional modeling techniques such as Partial Least Squares Regression (PLSR) to classes of models of higher complexity, such as Deep Learning (Figure 13).

As part of our research, we leverage the KSSL large spectral library and Deep Learning model (Convolutional Neural Network) to not only (i) predict exchangeable potassium in soil at a level of accuracy sufficient for agricultural or remediation applications but also to convey information on (ii) how uncertain individual predictions are and (iii) which features (spectral regions) of the input data drive individual predictions (Figure 14). All three characteristics are essential to inform decision-making and policies during large-scale crisis responses.
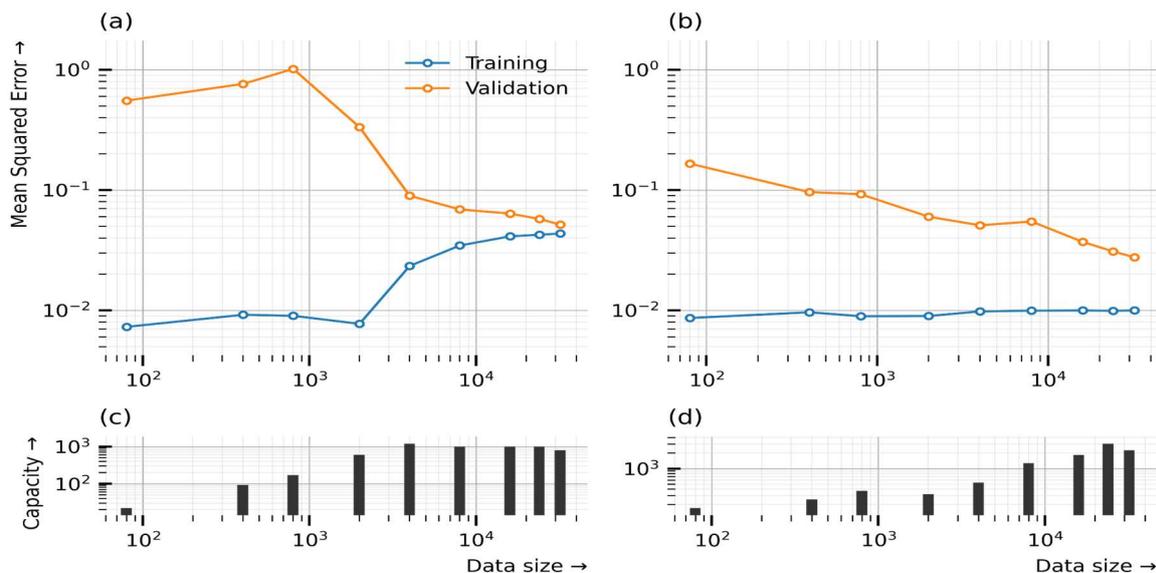
Figure 13. *"Learning Curve" to assess the ability of the Partial Least Square Regression (PLSR) and Convolutional Neural Network (CNN) models to leverage an increasing data regime through the inspection of the relationship between the performance of learning algorithms and the amount of data available. (a) PLS training and validation Mean Squared Error of log_{10}-transformed predicted variable and (c) PLS number of components when data size increases;(c) CNN training and validation Mean Squared Error and (d) CNN number of epochs when data size increases.*
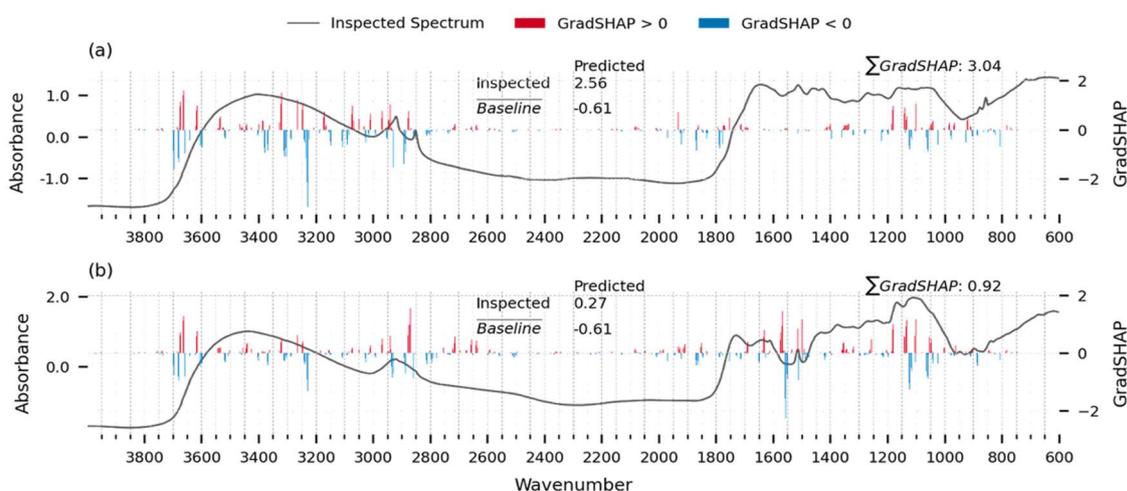
Figure 14. *Features importance of right predictions using GradientShap. (a) High and Right (b) Low and Right.*

Spatio-temporal clustering of contaminated agricultural parcels for efficient remediation in a post-accident situation

Under the Coordinated Research Project D1.50.19 on Remediation of Radioactive Contaminated Agricultural Land, collaboration between SWMCN Laboratory and SCK CEN aims to optimize agricultural remediation efforts to ensure food safety in the aftermath of a nuclear emergency. Given the large affected area, limited availability of labor and financial resources in the emergency phase, setting priorities of where, how and when to remediate are indispensable. Priority setting is particularly applicable when large agricultural areas are affected by long-lived radionuclides (e.g. radiocaesium) and the ambition is to return to normalcy as sustainably, efficiently and rapidly as possible.

Using operation research (OR) models, a spatio-temporal clustering approach was established to determine homogenous clusters of parcels, which should be remediated at the same time with the same technique. The timing of the remediation is based on a yearly budget constraint representing the limited economic resources available to deal with these problems. The results provide evidence that the proposed structured approach could help to optimize remedial decisions.

These novel findings show the positive impact these OR models could have on the remedial campaign (e.g. reduction of cost, waste production, local impact). The figures below show two extreme approaches, where the figure in the left proposes the optimal remediation actions for each individual field, resulting in a patchwork of remedial techniques and scattered priority sites. On contrary, the right figure proposes a single cluster of remediation and concentrated priority on the sites, although easily implemented it is sub-optimal. Based on the user preferences an approach balancing these two factors, feasibility, and optimality, will be proposed by the model returning a number of clusters and priorities to act on.
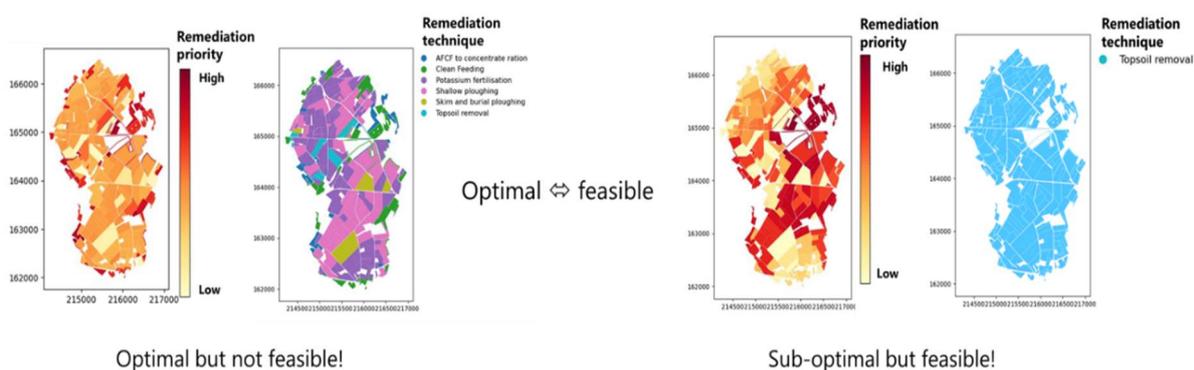
Future work will look at the impact of contaminated sediment movement in the landscape, taking into account the off-site effects parcels have in the landscape. This would allow us to further improve our decision support models, in determining a holistic approach to address remediation on a watershed level.

CAPACITY BUILDING & SERVICES

In 2021, the SWMCNL focussed on capacity building through the training and guidance of four PhD, three MSc students (through IAEA internships), two interns from seven countries in the use of nuclear and isotope techniques for climate-smart agriculture and nuclear emergency response. Further two virtual training courses were organized for in total 28 trainees from 6 countries on the use of cosmic ray neutron sensor technology and nitrogen-15 isotopes for improving agricultural water and soil fertility management.

Laboratory analyses

In 2021, 4659 samples were analysed for stable isotopes in the SWMCN Laboratory. Most analyses (i.e. 97%) were carried out for supporting Research and Development activities at the SWMCNL focused 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 an important tool for external quality control.

The 2021 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 has been 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 FAO/IAEA ^{15}N-enriched and three not ^{15}N-enriched test samples are included in one round of the WEPAL IPE (International Plant-Analytical Exchange) programme. A special evaluation report for 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 in addition to the regular WEPAL evaluation report. The participation fee for one round per year is covered by the IAEA.

In total seven stable isotope laboratories participated in the PT-round 2021: Africa (1): Morocco, Asia (1): Pakistan, Europe (3): Austria, Belgium and France and Latin America (2): Brazil.

Due to the COVID-19 situation the deadline for reporting of results was extended to 6 months. The laboratories, who were able to send the results, performed well regarding the C and N elementary analysis. However, as shown by the results below (Table 2), the ^{15}N and ^{13}C analyses were slightly below expectation, and less good than in other years. Nevertheless, it may be explained by the extended closure of the laboratories and the higher difficulty of isotope analysis. The lessons learned in these difficult times are of high importance to see what such extended closure may cause regarding the quality of isotope measurements.

Table 2. Number of results out of control limits.

Sample	δ ^{15}N	δ ^{13}C	C-elementary	N-elementary	Number of reporting labs
171	1	1	1	0	7
256	4	1	1	0	7
235 (Enriched ^{15}N)	2	1	1	0	6
130	2	2	0	0	7

GUIDELINES AND INFORMATION

Guidelines published in Journal of Environmental Radioactivity

In 2021, a special issue on 'Sampling, analysis and modelling technologies for large-scale nuclear emergencies affecting food and agriculture' was published in the Journal of Environmental Radioactivity. These guidelines provide background information, as well as generic non-country specific guidance about approaches for sampling and analysing soils, plants and food to scientists, policy makers and decision makers at different stages of the response phase during and after the

nuclear emergency. They are intended to promote standardized and efficient techniques in supporting large scale emergency response in food and agriculture. Specifically, they provide past studies and best practise examples on collecting samples, as well as promote an outlook and guidance on innovative methods. The work was conducted under the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, funded under an IAEA Coordinated Research Project (CRP) on "Response to Nuclear Emergency affecting Food and Agriculture" (CRP D1.50.15), from 2013 to 2019. The eleven guidelines can be downloaded from:

Journal of Environmental Radioactivity | Sampling, analysis and modelling technologies for large-scale nuclear emergencies affecting food and agriculture | ScienceDirect.com by Elsevier

Remediation of Radioactive Contamination in Agriculture: Next Steps and Way forward, NARO-FAO/IAEA Joint Symposium 2021 (Virtual).

For the 10th anniversary of the Fukushima Daiichi Nuclear Power Accident, the Joint FAO/IAEA Centre co-organized on 4 October 2021 the second NARO-FAO/IAEA Symposium, to disseminate the latest information on how to remediate radioactive contamination in agriculture. In total 96 participants from 14 Member States attended the symposium. Six out of ten presentations showed results from CRP D1.50.19.

Sharing our research progress and connecting with international researchers through the European Geosciences Union General Assembly 2021, Vienna, Austria

About 18172 scientists from 135 countries came together online for the European Geosciences Union (EGU) 2021 General Assembly held on 19-30 April 2021. The SWMCN Laboratory's activities were reported in 5 presentations covering topics on climate-smart nitrogen management, area-wide soil moisture monitoring, and remediation of radioactive contamination of agricultural land. The links to all contributions from the SWMCN Laboratory can be found in this annual report under the publication list, at the end of the SWMCNL contribution.

PUBLICATIONS

ALBINET, F., DERCON, G., EGUCHI, T. (2021). Deep Learning-based Soil Property Prediction for Remediation of Radioactive Contamination in Agriculture EGU General Assembly 2021. Abstract EGU21-4416. https://doi.org/10.5194/egusphere-egu21-4416

BOGENA, H., SCHRÖN, M., JAKOBI, J., NEY, P., ZACHARIAS, S., ANDREASEN, M., BAATZ, R., BOORMAN, D., DUYGU, B. M., EGUIBAR-GALÁN, M. A., FERSCH, B., FRANKE, T., GERIS, J., GONZÁLEZ SANCHIS, M., KERR, Y., KORF, T., MENGISTU, Z., MIALON, A., NASTA, P., NITYCHORUK, J., PISINARAS, V., RASCHE, D., ROSOLEM, R., SAID, H., SCHATTAN, P., ZREDA, M., ACHLEITNER, S., ALBENTOSA-HERNÁNDEZ, E., AKYÜREK, Z., BLUME, T., DEL CAMPO, A., DIMITROVA-PETROVA, K., EVANS, J. G., FRANCES, F., GÜNTNER, A., HERRMANN, F., IWEMA, J., JENSEN, K. H., KUNSTMANN, H., LIDÓN, A., LOOMS, M. C., OSWALD, S., PANAGOPOULOS, A., PATIL, A., POWER, D., REBMANN, C., ROMANO, N., SCHEIFFELE, L. M., SENEVIRATNE, S., WELTIN, G., AND VEREECKEN, H. (in review, 2021). COSMOS-Europe: A European Network of Cosmic-Ray Neutron Soil Moisture Sensors, Earth System Science Data [preprint], https://doi.org/10.5194/essd-2021-325.

CHENG, C.-H., MENYAILO, O., LEE, P.-C. (2021). Changes in soil organic carbon, nitrogen and phosphorus after abandonment of arable fields in Taiwan. PI046. European Confederation of Soil Science Societies, August 23-27, 2021, Geneva.

DERCON, G., ONDA, Y., BERESFORD, N. (2021). Sampling, analysis and modelling technologies for large-scale nuclear emergencies affecting food and agriculture. Special Issue in Journal of Environmental Radioactivity. Journal of Environmental Radioactivity | Sampling, analysis and modelling technologies for large-scale nuclear emergencies affecting food and agriculture | ScienceDirect.com by Elsevier

GIANESSI, S., POLO, M., STEVANATO, L., LUNARDON, M., SAID, H., WELTIN, G., TOLOZA, A., BUDACH, C., BIRO, P., FRANCKE, T., HEISTERMANN, M., OSAWALD, S.E., FULAJTAR, E., DERCON, G., HENG, L. K., BARONI, G. (2021). Assessment of a new non-invasive soil moisture sensor based on Cosmic-Ray Neutrons. Proceedings of IEEE 2021 International Workshop on Metrology for Agriculture and Forestry Pages 290-294, November 3-5, 2021, Trento.

HEILING, M., SHORAFA, M., MIRKHANI, R., WILLEMS, E., TOLOZA, A., RESCH, C., HENG, L.K., DERCON, G. (2021) Influence of different nitrogen inhibitors on maize yield. EGU General Assembly 2021. Abstract EGU21-9859. https://doi.org/10.5194/egusphere-egu21-9859

LIN, Y.-H., LEE, P.-C., MENYAILO, O.V., CHENG, C.-H. (2021). Changes in soil organic carbon concentration and stock after forest regeneration of agricultural fields in Taiwan. Forests, 12 (9):1222. https://doi.org/10.3390/f12091222.

MAMAI, W., MAIGA, H., BIMBILÉ SOMDA, N.S., WALLNER, T., MASSO, O.B., RESCH, C., YAMADA, H., BOUYER, J. (2021) Does TapWater Quality Compromise the Production of Aedes Mosquitoes in Genetic Control Projects? Insects, 12, 57. https://doi.org/10.3390/insects12010057

MASYAGINA, O.V., EVGRAFOVA, S.Y., MENYAILO, O.V., MORI, S., KOIKE, T., PROKUSHKIN, S.G. (2021). Age-dependent changes in soil respiration and associated parameters in Siberian permafrost larch stands affected by wildfire. Forests, 12 (1): 107. https://doi.org/10.3390/f12010107.

MBAYE, M., SAID, H., FRANZ, T., WELTIN, G., DERCON, G., HENG, L. K., FULAJTAR, E., STRAUSS, P., RAB, G., NDIAYE, M. (2021) Deep learning approach for calibrating Cosmic-Ray Neutron Sensors (CRNS) in area-wide soil moisture monitoring. EGU General Assembly 2021. Abstract EGU21-9803. https://doi.org/10.5194/egusphere-egu21-9803

MBAYE, M., SAID, H., FRANZ, T., WELTIN, G., TOLOZA, A., DERCON, G., HENG, L. K., FULAJTAR, E., STRAUSS, P., RAB, G., NDIAYE, M. (2021). Deep learning approach for calibrating Cosmic-Ray Neutron Sensors (CRNS) in area-wide soil moisture monitoring. Technical Meeting on Artificial Intelligence for Nuclear Technology and Applications, October 25-29, 2021, Vienna. https://conferences.iaea.org/event/245/overview.

MENYAILO, O. V., CHENG, C.-H., MATVIENKO, A. (2021). Effects of temperature, N and P application on the priming effects in soils under spruce and temperature grassland. European Confederation of Soil Science Societies, August 23-27, 2021, Geneva.

MIRKHANI, R., GHAVAMI, M.S., AHMADI, E., MOGHISEH, E. (2021) Influence of nitrification inhibitor (Nitrapyrin) on winter wheat yield. EGU General Assembly 2021. Abstract EGU21-9684. https://doi.org/10.5194/egusphere-egu21-9684

SAID, H., MBAYE, M., HENG, L. K., FULAJTAR, E., WELTIN, G., FRANZ, T., DERCON, G., STRAUSS, P., RAB, G., SAUD AL-MENAIA, H., NDIAYE, M. (2021) High-resolution soil moisture mapping through the use of Cosmic-Ray Neutron Sensor and Sentinel-1 data for temperate and semi-arid environments. EGU General Assembly 2021. Abstract EGU21-9688. https://doi.org/10.5194/egusphere-egu21-9688

SAID, H., MBAYE, M., HENG, L. K., WELTIN, G., FRANZ, T., DERCON, G., TOLOZA, A., FULAJTAR, E., STRAUSS, P., RAB, G. (2021). High-resolution soil moisture retrieval using C-band radar Sentinel-1 and Cosmic-Ray Neutron Sensor. Proceedings of IEEE 2021 International Workshop on Metrology for Agriculture and Forestry Pages 221-224, November 3-5, 2021, Trento, Italy.

TIEFENBACHER, A., WEIGELHOFER, G., KLIK, A., MABIT, L., SANTNER, J., WENZEL, W., STRAUSS, P. (2021). Antecedent soil moisture and rain intensity control pathways and quality of organic carbon exports from arable land. Catena, 202, 105297. https://doi.org/10.1016/j.catena.2021.105297

VANTYGHEM, M., BEELEN, E., MERCKX, R., HOOD-NOWOTNY, R., HEILING, M., RESCH, C., DERCON, G. (2021). Unraveling carbon dynamics in banana mats through a ^{13}C labeling experiment. 18th Stable Isotope Network Austria (SINA) Meeting. November 11-12, Tulln, Austria.

VANTYGHEM, M., MERCKX, R., STEVENS, B., HOOD-NOWOTNY, R., SWENNEN, R., DERCON, G. (2022). The potential of stable carbon isotope ratios and leaf temperature as proxies for drought stress in banana under field conditions. Agricultural Water Management, 260, 107247. https://doi.org/10.1016/j.agwat.2021.107247

WELLENS, J., RAES, D., FERERES, E., DIELS, J., COPPYE, C., ADIELE J.G., EZUI, K.S.G., BECERRA, L.-A., SELVARAJ, M.G., DERCON, G., HENG, L.K. (2022). Calibration and validation of the FAO AquaCrop water productivity model for cassava (Manihot esculenta Crantz). Agricultural Water Management, 263, 107491. Calibration and validation of the FAO AquaCrop water productivity model for cassava (Manihot esculenta Crantz) - ScienceDirect

AN UPDATE ON THE RENUAL INITIATIVE: THE FAO/IAEA AGRICULTURE AND BIOTECHNOLOGY LABORATORIES

The modernization of NA laboratories began in 2014 with the Renovation of the Nuclear Applications Laboratories (ReNuAL) project, which consisted of new building construction to provide modern laboratory space, the acquisition of new laboratory equipment and infrastructure upgrades. A follow-up to ReNuAL, called ReNuAL Plus (ReNuAL+), provided for further construction and modernization of laboratory facilities. As a result of the ReNuAL/ReNuAL+ initiative, two new buildings were built (Insect Pest Control Laboratory building and Yukiya Amano Laboratories) housing a total of four NA laboratories, and a new linear accelerator facility was added to the existing Dosimetry Laboratory.

Yukiya Amano Laboratories Building Officially Opened

Director General Grossi and Austrian Foreign Minister Alexander Schallenberg formally opened the new Yukiya Amano Laboratories (YAL) building on 5 June 2020. The event marked the completion of all major construction begun since the launch of the ReNuAL/ReNuAL+ initiative. The YAL building is home to the Animal Production and Health Laboratory, Food Safety and Control Laboratory, and the Soil and Water Management & Crop Nutrition Laboratory. All three laboratories have moved into the building and are fully operational.

ReNuAL2

In September 2020, Director General Grossi briefed Member States on plans for addressing the NA laboratories not yet renovated under the ReNuAL/ReNuAL+ project. The DG outlined the Agency's approach for the final phase of the project, calling it ReNuAL2, which will complete the modernization of the laboratories at Seibersdorf and fulfil the vision of providing laboratory facilities to meet current and emerging needs.

The three main elements of this phase are 1) construction of a new laboratory building (called FML2) to serve as home for three remaining NA laboratories; 2) refurbishment of the Dosimetry Laboratory in its current location; and 3) replacement of aging greenhouses.

The New Laboratory Building

The new facility will house the Terrestrial Environment and Radiochemistry Laboratory, the Plant Breeding and Genetics Laboratory, and the Nuclear Science and Instrumentation Laboratory. The detailed design phase for this building has been completed and bids for the construction contract are currently under review. The target date for lunching construction is first half of 2022 with completion projected for early 2024.

The Dosimetry Laboratory Refurbishment

The Dosimetry Laboratory (DOL) supports radiation dosimetry through the provision of services to IAEA Member States, including dosimetry calibration and dose auditing. As part of the ReNuAL/ReNuAL+ initiative, this laboratory has increased and enhanced its support for Member States by obtaining a new Linear Accelerator Facility, opened in June 2019.

Located in one of the newest wings of the existing NA laboratory facilities in close proximity to the new LINAC facility, this laboratory will undergo refurbishment in its current location.

The Greenhouses

The IAEA's existing greenhouses are over 30 years old and approaching the end of their service life. The new greenhouses will provide expanded space and modern facilities, including a separate climate-controlled hot greenhouse and a climate-controlled growth chamber, providing new on-site capacities for research and development.

The greenhouses are essential to the work of the Plant Breeding and Genetics Laboratory, the Soil and Water Management and Crop Nutrition Laboratory, and the Food Safety and Control Laboratory.

ReNuAL Resource Mobilization Update

A total of 42 Member States (and some individuals and institutions) provided over €39.7M to the ReNuAL/ReNuAL+ project and have been recognized on the donor wall installed in the new Insect Pest Control Building.

27 Member States (and the FAO) have already announced contributions to final phase of the project, ReNuAL2, since the 64th General Conference in September 2020. These and all subsequent ReNuAL2 contributions will be recognized on a new donor display that will be permanently installed in the new FML2 laboratory building upon its completion.

During the March Board of Governors, Member States announced a pledge of 6.7 M Euro for construction of the new laboratories building in 2022. The project team is moving to mobilize resources for the new greenhouses in the coming months.