

# Agriculture and Food Security Project List for 2024 Recruitment

## Contents

New potent antiparasitic agents against livestock resistant roundworms.....	3
Semi-dry cell agriculture of low carbon-footprint cultured meat.....	4
Coronaviruses in UK wildlife.....	5
Engineering chimeric antimicrobials with multiple mode of action for advanced food protection.....	6
Developing genomic epidemiology frameworks for pathogenic mycobacteria in animals. ....	7
Understanding the role of <i>Porphyromonas asaccharolytica</i> in ovine footrot.....	9
The genetic and developmental basis of salinity tolerance in teleost fishes.....	10
Using bacterial exometabolites to redesign plant roots .....	12
Manipulating targeted genes to improve barley resilience: characterising novel environmental stress regulators. ....	13
Development and assessment of a novel sensitive method for the genetic analysis of complex traits.....	15
Investigating the mechanisms of pH homeostasis linked temperature stress response in plants.....	16
The identification of novel methods for re-emerging <i>Enterococci</i> pathogen management in intensive poultry farming.....	18
How mechanosensing drives root penetration in hard soil? .....	19
Smart adsorbent materials to mitigate antimicrobial resistance in dairy farm wastewaters (SAM-FARM).....	20
How a gaseous hormone is made, mobilized and mediates stress responses in plant tissues. ....	22
Characterisation and validation of Cougar-derived candidate genes for resistance to <i>Septoria tritici</i> blotch (STB) in wheat.....	24
Enhancing heat tolerance in wheat from wheat wild relatives to improve food security. ....	25
Paramyxovirus spillover between humans and livestock - potential for a new pandemic?.....	27
One Health surveillance approaches to fight AMR using Artificial Intelligence and big data mining .....	28
Investigating the role of cytokinin in regulating drought tolerance. ....	29
Space Farming: Optimising resource use efficiency in Bioregenerative life support systems (BLSS).....	31
Phenotypic and molecular characterisation of rice <i>axr4</i> mutants .....	32
Environmental bioremediation using predatory bacteria – taking the first steps towards applications of <i>Bdellovibrio</i> against pathogens in farm waste.....	34

Microbiomes of beneficial arthropods in agricultural systems and their influence on biocontrol .....	35
Harvesting novel alleles from the <i>Triticum timopheevii</i> genome for bread wheat improvement .....	37
Defend or Welcome: Evolution and regulation of plant cell walls to control microbial colonisation. ....	38
Environmental pollen resilience:- understanding the impact of heat and light stress on pollen development to maintain yield under environmental stress.....	40
Metaphenomics as a tool for assessing soil health.....	41
Methane microbiology .....	43

## New potent antiparasitic agents against livestock resistant roundworms

Project Supervisor: Hany Elsheikha

**School:** Veterinary Medicine and Science

**Lab Rotation Description:** The rotation project aims to perform phenotypic screening using roundworm larval motility inhibition assay against analogues of the lead (most effective) molecules of proven antiparasitic activities.

- Week 1: Introduction to mineral status, nutritional analysis, and benefits from willow trees. (Nottingham Vet School: NUVetNA – Lab B47)
- Week 2: Conduct virtual screens of chemical databases to identify compounds with a similar chemical structure and functionality to the identified natural lead compounds. (Nottingham Trent University School of Science and Technology: Chemistry – Lab NSRC113 ).
- Week 3-6: Perform microscopy-based phenotypic screens to identify the antiparasitic efficacy of newly synthesised analogues of the lead compounds. (Nottingham Vet School: Parasitology Lab B54)
- Week 7-9: Analysis of the cytotoxic properties of the newly synthesised analogues using established in vitro assays. (Nottingham Vet School: Parasitology Lab B54).

The research project will provide the student with a first-hand mentored research experience on the process of therapeutic discovery and screening in the emerging researched field of antiparasitic drug discovery.

**Location:** Sutton Bonington Campus; Clifton Campus;

**Full project description:** Livestock farming is facing an enormous threat from the emergence of parasites that are resistant to all commercially available antiparasitic drugs. Gastrointestinal roundworm infections of ruminants impose a significant threat to livestock health and productivity. Despite advances in controlling parasite infections in livestock using antiparasitic drugs, parasite infections remain an important challenge to a sustainable agricultural economy, due to the rapid and high rate of resistance development. Ensuring sustainable agricultural and livestock production systems is essential for addressing the threat of food insecurity. The proposed PhD research project “New potent antiparasitic agents against livestock resistant roundworms” led by Hany Elsheikha from Nottingham Vet School, UoN aims to develop novel, efficient, specific, and safe anthelmintic drug candidates to improve parasite control practice and offer efficient alternatives in the difficult-to-treat situations (e.g., infection with multi-resistant parasites).

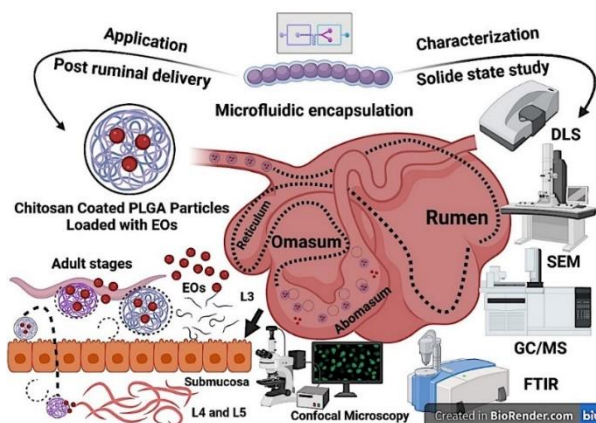
Research on the antiparasitic properties of medicinal plants has garnered a significant interest because they present fewer side effects and are less likely to lose efficacy to resistant parasites compared to antiparasitics currently in use. Due to the nutritional and medicinal properties of willow tree leaves (*Salix* spp), we assessed the willow’s potential for novel antiparasitic drugs. We profiled the chemical constituents of methanolic extract of willow leaves using GC/MS and identified 22 phytochemicals in the willow extract. We then utilized a microscopy-based phenotypic screens to identify inhibitors of motility from the 22 isolated phytochemicals. Two phytocomponents (neophytadiene and pentadecanal) were identified as potent inhibitors of the parasite larval motility with inhibition rates of 88.44% and 64.48% at the concentration of 500 µg/mL and 125 µg/mL, respectively, compared with the positive control treatment. Scanning electron microscopy showed morphological changes such as damage of the outer layer of the treated parasite larvae. None of the tested concentrations (125, 250 and 500 µg/mL)

was cytotoxic to bovine intestinal cells in vitro, only pentadecanal induced a significant reduction of cell viability at the highest dose 500 µg/mL.

Our studies showed that neophytadiene and pentadecanal have antiparasitic activity against several roundworms, suggesting a potential cross-roundworm compound class and provide a platform for further chemical derivatization of a potent chemical scaffold against roundworms. The establishment of significant in vitro antiparasitic efficacy of neophytadiene and pentadecanal provides invaluable resources for the discovery of new antiparasitic compounds and contribute to the utilization of the waste from willow cultivation. The aims of this PhD are to: (1) determine the molecular targets of neophytadiene and pentadecanal by performing reverse docking experiments between these lead compounds and roundworm putative target proteins, (2) elucidate the mechanism of actions of neophytadiene and pentadecanal, and (3) perform structure-activity relationship analysis to identify analogues with better anti-parasitic and safety properties.

The project findings will accelerate the rational design of novel therapeutics for gastrointestinal roundworm infections of livestock. We will employ an integrative strategy involving molecular biology, biochemistry, metabolic profiling, structural and cell biology to discover new antiparasitic targets, elucidate the molecular mechanisms of actions of the lead molecules, and analyse potential adverse drug reactions and drug toxicity.

**Full project location:** Sutton Bonington Campus;



## Semi-dry cell agriculture of low carbon-footprint cultured meat

**Project Supervisor:** James Dixon

**School:** Pharmacy

**Description:** The lab rotation will demonstrate the initial proof-of-concept that myogenically programmed cells in hydrogels can be proliferated and differentiate in the core-shell system semi-dry. Cell number, metabolism, viability, myogenic differentiation (QPCR and immunostaining) will be conducted with potential for electrical stimulation of the generated muscle. Different hydrogel formulations will be tested examining moisture retention, nutrient and gaseous transport to the core muscle.

You will culture and program stem cells (iPSCs, MSCs and skin-derived cells from tissue) programmed with non-viral gene delivery of transcription factors which will trigger the myogenic program and generate myogenic progenitor and terminally differentiated skeletal muscle cells. These will be cultured conventionally or semi-dry in hydrogels by simple or core-shell fabrication. Parameters of the core and shell materials will be examined which will progress on to the PhD study where other cell types (adipose, connective tissue) will be programmed and integrated to generate an authentic CM platform.

**Location:** University Park;

**Full project description:** Background: Global demand for meat is estimated to double by 2050, however meeting the increased demand under current systems is not sustainable. The production of cultivated meat as an alternative source of high-quality animal proteins for human consumption has gained traction in recent years due to the considerable progress in the technologies underpinning the manufacturing of this novel product. The recent approval of CM products in Singapore and US provide an unprecedented opportunity for accelerating R&D efforts to develop commercially viable options that will satisfy consumer demand for meat globally. Although proof-of-concept products have recently been reported, the commercial viability of these is questionable due to limitations in scale and high production costs. There are significant technical challenges that need to be addressed for CM to become an attractive proposition for consumers.

**Approach:** This PhD will focus on developing semi-dry cell culture for growth and differentiation of CM. The programme of work builds on gains in state-of-the-art gene programming and cell culture technology for the cell component. The student will develop hydrogel fabrication via extrusion additive manufacture and bioreactor design to generate rapid meat from edible, scalable, environmentally sound and fully deployable platform. This will be applied to the main meat groups (poultry, beef, lamb, pork) with the aim to generate kg/hour rates in a week maturation single intervention process prior to next-stage industrialisation. Novel concepts will be used here that are not currently being exploited by the CM sector, and has the scope to be transformative in generating alternatives to animal-derived muscle for food. Core-shell extrusion and textile approaches will be used to fabricate muscle fibres coated with protective, edible and nutrient/gas-diffusing gels to retain viability and moisture to expand and generate muscle in the developed semi-dry culture system. Stratified anisotropic muscle will be generated de novo in this process, bypassing the need for fermentation and conventional culture industrialisation. This would be transformative for the CM concept.

**Location:** The project will be carried out in the new state-of-the-art Biodiscovery institute at University Park (James Dixon, Jing Yang) and the School of Biosciences at Sutton Bonington Campus (Ramiro Alberio). The use of animal cell culture and genetic programming in this application will be transformative and is likely to have significant commercial value and interaction with stakeholders. Presently Dixon has funding for bone and fat reconstructive technologies in humans using gene delivery in the Dixon group and this PhD could add impact and synergy to these projects reciprocally.

**Techniques and opportunities:** The project will use additive manufacturing techniques, mechanical testing, texture analyses, nutrient/taste profiling, cell/stem cell culture, QPCR, immunolabelling, flow cytometry, single cell RNAseq, vector design and construction, gene delivery, scaffolds, tissue engineering, electrical stimulation, with potential to demonstrate authentic muscle generation by in vivo testing.

**Full project location:** University Park; Sutton Bonington Campus;

[Coronaviruses in UK wildlife](#)

**Project Supervisor:** Rachael Tarlinton

**School:** Veterinary Medicine and Science

**Description:** The lab rotation will consist of a mix of lab work (PCR and sequencing, cell culture) and introductory bio-informatics (sequence analysis), analysing a stored bank of samples for the presence of coronaviruses (novel and known) in UK wildlife species. No prior experience with computing required as we can teach that but it would be good to

have a basic grasp of things like excel spreadsheets. You will be joining the "One Virology" group of 5 academics and 20 PhD and postdoctoral scientists based at the vet school in Sutton Bonington and will participate in the groups activities

**Location:** Sutton Bonington Campus;

**Full project description:** The ongoing adaptation of SARS-CoV-2 to people has resulted in repeated spill-over into a wide variety of animal species with the establishment of a new reservoir for the virus in white tailed deer in North America one of the more serious outcomes. It is becoming increasingly clear that the species range of SARS-CoV-2 is very dependent on which strain of the virus is currently circulating in the human population and is not very stable. Work we and others conducted in 2020-21 indicated a lack of widespread circulation of the virus in European Wildlife, however there are increasing reports of positive animals (including a confirmed outbreak in fallow deer). This project will focus on going work on the species range of SARs-CoV-2 and will consist of a mix of epidemiology work (coronavirus monitoring in samples from wild animal species, including novel wildlife coronaviruses), sequencing work of any viruses found and cell culture work to establish cell culture systems (or tissue explant systems) for European wildlife species for coronavirus infection studies. Many of our species do not have basic laboratory tools available, like cell-lines for doing even basic work on receptor tropism or species range of coronaviruses and this work will seek to establish that for common species and viruses, including genomic and transcriptomic sequencing of responses to coronaviral infection in these species.

**Full project location:** Sutton Bonington Campus;

[Engineering chimeric antimicrobials with multiple mode of action for advanced food protection](#)

**Project Supervisor:** Boyan Bonev

**School:** Life Sciences

**Description:** This is a molecular design project. The rotation period aims to provide an introduction to the partner labs and the methodology that will be used in the project. These include a combination of biochemistry, microbiology and biophysics, such as biosynthetic production of complex biological molecules, compound purification and characterisation, antibiotic susceptibility testing and fluorescence spectroscopy. It offers an overview of the early stages of molecular design and architectural considerations for target selectivity and specificity, structural considerations for antimicrobial and antibiofilm activity. You will investigate the biological function and molecular interactions between lead compounds ahead of designing target-informed antimicrobials with modular and enhanced functionality. To do this, the following step will be undertaken: w1- introduction to the lab and project; w2,3: production and purification of lead compound nisin (E234); w4,5: activity testing against bacterial strains using agar diffusion and broth dilution assays; w6,7: production and purification of molecular target, lipid II; w8,9: fluorescence dequenching assays of molecular recognition of target lipid II by nisin.

**Location:** QMC;

**Full project description:** Access to sufficient, nutritious and safe food is a major challenge for a growing global population. While sustainable production is reaching the planetary limits, food spoilage leads to losses of nearly a third of the food supply during transportation, storage and processing. A major part of food waste is due to spoilage

through contamination. Food production is a multi-\$bn industry with tight margins and the food supply is critically dependent on reducing waste. To achieve this, we seek to develop effective antimicrobials with no perceivable taste, smell or colour.

Antimicrobial compounds are widely used in food preservation, veterinary medicine, packaging and device and carrier protection to prevent and remove biofilms. The molecular architecture of antimicrobials with such diverse application is challenging. Our primary objective is to engineer, characterise and validate compounds with antimicrobial activity, surface adhesion modulating properties (antibiofilm activity), which are paralleled by low toxicity and safety in the food chain. To achieve this, we combine building blocks from different molecules that confer different properties to the chimeric compound. We combine molecular fragments that confer powerful killing action with block that target specific molecular targets present only in bacteria that make them safe for human consumption.

This is a structure-informed molecular design project. The overall aim is to develop chimeric antimicrobials with multiple mode of action [Hyde et al. PNAS 2006] and broader spectrum of activity, capable of interfering with bacterial division and simultaneously with cell envelope formation, as well as disrupting bacterial plasma membranes [Hasper et al., Science 2006; Bonev et al., FASEB J 2004]. Selectivity in the design relies on recognition of highly specific molecular targets [Breukink et al., Science 2004] unique to bacteria, which are refractive to alteration and resistance [Ling et al., Nature 2015; Bayley et al., Bacterial Resistance to Antibiotics 2019]. Lead compounds will be engineered, produced, tested and optimised for favourable antimicrobial properties and directed evolution will be used to assess any routes to bacterial adaptation.

We aim to use nature-inspired peptidoglycan-targeting epitopes with receptor-independent membrane-disrupting segments. Your challenge will be to combine target recognition epitopes from lipid II-targeting lantibiotics such as nisin, or teixobactin, with enhanced pore-forming peptides, such as magainin and PGLA. We will make the chimeras biosynthetically and will assess their antimicrobial and antibiofilm action, as well as their ability to suppress antibiotic resistant strains. We will use structural approaches to gather experimental evidence of the molecular interactions and target engagement by a) the recognition epitope alone, b) of a chimera with the membrane disruptor and c) the membrane disrupting compound alone as control. We will use fluorescently tagged antimicrobials and superresolution microscopy to will investigate target presentation and localisation on bacterial cells to understand the fundamental processes of peptidoglycan biosynthesis.

**Full project location:** QMC;

[Developing genomic epidemiology frameworks for pathogenic mycobacteria in animals.](#)

**Project Supervisor:** Conor Meehan

**School:** School of Science and Technology (NTU)

**Description:** The purpose of the rotation mini project will be to train the student in the current state of the art molecular epidemiology pipelines for mycobacteria. There are standard whole genome sequencing assembly pipelines for M. tuberculosis human lineages and the student will be trained in the theory of these processes and given test datasets to learn the practical computer skills for applying them. Once the student is

familiar with the processes, we will investigate their applicability for the animal variants of *M. tuberculosis*. Public datasets will be used to assess the pipelines for their accuracy in reconstructing recent transmission of *M. tuberculosis* variants *bovis* and *orygis*, similarly to what was done previously for lineage 4 (see Meehan et al, *EBioMedicine*, 2018). This extended training will include introductions to phylogenetics, including Bayesian approaches, as well as molecular epidemiology and visualisation of outputs.

Skills learned by the student in rotation: UNIX, R, genome assembly, comparative genomics, introduction to Bayesian phylogenetics, transmission clusters and reconstruction, genetics of mycobacteria. All of these are transferable skills and highly sought after in future employment opportunities.

**Location:** Clifton Campus;

**Full project description:** Infection of both agricultural and wildlife animals with different species of mycobacteria is a large burden, both in terms of animal health and associated economic costs. Two of the primary mycobacteria infecting such animals are *Mycobacterium tuberculosis* variants *bovis* and *orygis* infecting cattle, sheep, deer, badgers and rhino and *Mycobacterium avium* subspecies *paratuberculosis* infecting cattle, sheep, goats, rabbits, deer and bison.

The frameworks used for tracking mycobacterial infections are almost exclusively built around tracking *Mycobacterium tuberculosis* in humans. They rely on whole genome sequencing data from the pathogens which are then compared to see how many mutations each pair differ by. If this difference is less than five single nucleotide polymorphisms, the two isolates are said to be in a transmission cluster together. However, these approaches have not been tailored for any other mycobacteria, who have different genome sizes and mutation rates.

This project aims to create gold standards for tracking transmission of animal-associated pathogenic mycobacteria similar to those in place for *M. tuberculosis* in humans. This will be achieved through various work packages including:

- Analysis of mutation rates and genome comparison approaches for *M. tuberculosis* animal variants and *M. avium* subsp *paratuberculosis*
- Create adapted computational frameworks for undertaking track and trace of these pathogens using clinically derived whole genome samples
- Extension of these pipelines to look for drug resistance and virulence factors
- Use these pipelines to better understand the transmission of pathogenic mycobacteria between animals, with a particular focus of transmission corridors between agricultural and wildlife animals.

The project will be almost wholly computational and develop the student in skills such as:

- Bioinformatics pipeline construction
- Comparative genomics
- Molecular epidemiology, in particular Bayesian phylogenetics
- Clinical bacteriology, in particular mycobacteriology
- Scripting languages such as python, UNIX and R



Prior bioinformatics training is desired but not essential as all relevant training will be provided during the project.

**Full project location:** Sutton Bonington Campus; Clifton Campus;

[Understanding the role of \*Porphyromonas asaccharolytica\* in ovine footrot](#)

**Project Supervisor:** Rachel Clifton

**School:** Veterinary Medicine and Science

**Description:** The nine-week lab rotation will provide a taster of both laboratory and bioinformatics methods involved in the project. The student will gain experience of culturing two stock strains of *P. asaccharolytica* that were previously isolated from sheep feet. They will then extract DNA from these strains and submit for whole genome sequencing. The student will then be able to perform some initial bioinformatics analyses on the resulting sequence data, for example genome assembly and alignment to reference genomes. Whilst awaiting genome sequence data the student will begin development of co-culture methods for *P. asaccharolytica*, *Fusobacterium necrophorum* and *Dichelobacter nodosus*. They will assess growth of each organism on different types of solid media to determine which media would be most suitable for each pairwise combination. They will then pilot this approach by inoculating the selected media with these pairwise combinations and making visual assessments of growth.

**Location:** Sutton Bonington Campus;

**Full project description:** Footrot is a polymicrobial disease of sheep where bacterial invasion of the interdigital skin causes inflammation and separation of hoof horn. The resulting lameness has severe impacts on animal welfare as well as weight gain, wool growth, fertility, and longevity; these impacts are evident worldwide and across all farming systems.

There are key knowledge gaps regarding footrot pathogenesis. Research suggests that increased abundance of *Porphyromonas asaccharolytica* predisposes sheep to disease, however the mechanism for this is unknown. Studies of one strain of *P. asaccharolytica* from humans revealed expression of proteases that degrade extracellular matrix. Footrot pathogenesis involves separation of hoof horn from underlying tissues, therefore the student will test the hypothesis that sheep strains of *P. asaccharolytica* produce proteases that contribute to tissue destruction.

The related species *Porphyromonas gingivalis* is a key pathogen in periodontal disease and is known to interact with other pathogens to influence disease pathogenesis. Therefore, the second hypothesis the student will test is that *P. asaccharolytica* interacts with two known footrot pathogens, *Dichelobacter nodosus* and *Fusobacterium necrophorum*. They will use a novel liquid extraction surface analysis mass spectrometry (LESA-MS) approach to identify metabolites involved in bacterial signalling between these species.

Objective 1: Development of a collection of *P. asaccharolytica* isolates.

Foot swabs will be collected from sheep feet and cultured anaerobically to isolate *P. asaccharolytica* strains. These will be stored for use in subsequent objectives.

Objective 2: Whole genome sequencing and comparative genomics of sheep isolates of *P. asaccharolytica*.

DNA extracted from *P. asaccharolytica* strains will be submitted for whole genome sequencing using Illumina sequencing. Genomes will be assembled and aligned to available reference strains and genome annotation will be performed to identify potential virulence factors. Comparative genome analysis of the sheep strains and reference strains will be performed to identify orthologous gene clusters.

Objective 3: Comparing protease expression in sheep isolates of *P. asaccharolytica* to the type-strain DSM20707

Protease expression will be examined for a subset of the sheep isolates obtained in obj.

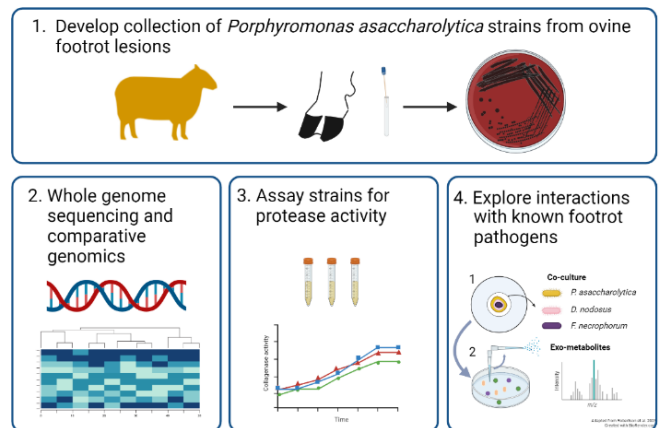
1. The type-strain DSM20707 will be assayed alongside the sheep strains for comparison. Cell suspensions and cell-free supernatants will be tested for collagenase activity and cell-free supernatants will be tested for general protease activity using fluorescein (FITC)-labelled casein as a substrate. Because separation of hoof from underlying tissue is an important feature of footrot pathogenesis, proteolytic degradation of hoof by *P. asaccharolytica* strains will be assayed.

Objective 4: Exploring interactions between *P. asaccharolytica* and known footrot pathogens.

Comparison of bacterial growth on solid media of single isolates and pairwise co-cultures

of *P. asaccharolytica*, *D. nodosus* and *F. necrophorum* will be used to identify and characterize interactions between these species. To gain mechanistic understanding of bacterial signalling between the three species, LESA-MS will be used to compare secreted metabolites from single and co-cultures. Identification of these metabolites from reference databases will be used to infer mechanisms underlying observed bacterial interactions.

Through these objectives, this project will identify key mechanisms by which *P. asaccharolytica* contributes to footrot pathogenesis.



**Full project location:** Sutton Bonington Campus; University Park;

[The genetic and developmental basis of salinity tolerance in teleost fishes.](#)

**Project Supervisor:** Andrew MacColl

**School:** Life Sciences

**Description:** The aim of this project will be to select a gene linked to salinity tolerance in stickleback, to knock out and knock down the gene within zebrafish embryos and to characterise the effect of its loss on salinity tolerance. Candidate genes will be selected by cross-referencing existing stickleback and wider literature on teleost salt tolerance. Using genome editing approaches, the student will generate a zebrafish G0 mosaic mutant by CRISPR/Cas9 and also knock the same gene down by CRISPR interference (CRISPRi), which uses a catalytically inactive form of Cas9 (dCas9). The extent of knockout/knockdown will be quantified by qPCR and/or in situ hybridisation. The consequence of loss of gene function on developing embryos will be determined using

confocal and/or lightsheet fluorescence microscopy of transgenic zebrafish embryos. The salt tolerance phenotype generated by each approach will be directly compared.

During the project the student will learn how to perform microinjection of zebrafish embryos to facilitate gene knockout by Crispr/Cas9 and gene knockdown by CRISPRi, quantification of gene expression by qPCR and/or in situ hybridisation, confocal and/or lightsheet fluorescence microscopy of transgenic zebrafish embryos and associated molecular biology techniques. The student will also learn how to interrogate genomic databases including Ensembl.

**Location:** QMC;University Park;

**Full project description:** Transfers of fish between freshwater and saltwater in aquaculture are a significant source of economic loss through stress but our understanding of salt tolerance in fishes is incomplete. A fuller understanding of this important topic could be achieved by studying natural variation in model species. However, there are no fish species that combine significant natural variation in salinity tolerance with cutting-edge research tools. This interdisciplinary project will couple two model species to achieve a greater understanding of the evolution of salt tolerance in teleosts.

Genomic and morphological changes associated with salinity change have been well described in stickleback, an ecological model species, but studies have not causally connected genotype and phenotype to achieve a functional genomic understanding, because the necessary research tools are less well-developed. In contrast, the availability of cutting edge tools means that the connection between genotype and phenotype through development is much more amenable to study in zebrafish, but they lack knowledge of genomic variation in natural populations and only occur in freshwater.

The goal of this research is to synergistically connect the different strengths of stickleback and zebrafish, to understand how ancestrally marine fish are able to adapt to freshwater environments. The student will borrow tools used in zebrafish development studies, including fluorescent transgenic labelling and live confocal microscopy to follow development in embryos, gene knockdown methods including CRISPR interference and gene editing to generate mutants, achieved via microinjection of stickleback and zebrafish embryos. The student will address two main questions:

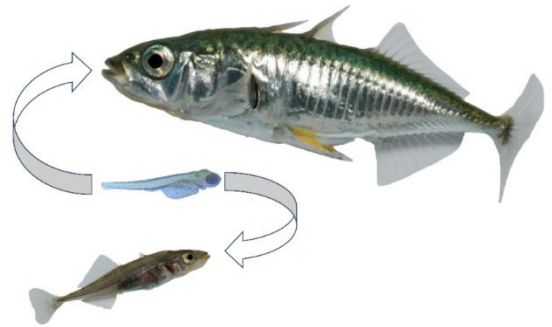
(1) How are variations in gene expression during development linked to phenotypic consequences? The student will identify strong candidate loci for freshwater adaptation by cross-referencing stickleback marine-freshwater divergent loci with the wider literature on freshwater adaptation and developmental abnormality in teleosts. Obvious possibilities include *ATP1a1*, a gene likely involved in ion transport across plasma membranes and *Wnt7b*, a gene widely involved in development.

(2) Can patterns in (1) explain a novel developmental disorder associated with freshwater colonization in stickleback? Marine stickleback raised in 0 ppt salinity often show developmental abnormality, reminiscent of zebrafish scoliosis. Such a phenotype would be likely to suffer reduced fitness in natural conditions. The student will characterise the development of the phenotype, including when it first appears, which tissues and organs it affects, and the sensitivity to concentrations of different salt/freshwater ions. The student will perform RNAseq to quantify differences in gene expression in appropriate tissues/time points, to identify underlying candidate gene. These will be cross-referenced with (1), above.

The student will investigate the best of these candidates during development in salt/freshwater stickleback, by quantifying gene expression differences, mapping gene expression by whole-mount in situ hybridization (WISH) in early larval stages and in specific organs in adults, gene knock-down with CRISPRi and CRISPR/Cas9-mediated targeted mutagenesis in sticklebacks to gain further understanding of the phenotypic differences regulated by these genes.

This project will contribute to a full characterisation of the genes that enable adaptation to freshwater, informing breeding programmes in a wide variety of teleost aquaculture species.

**Full project location:** University Park;QMC;



[Using bacterial exometabolites to redesign plant roots](#)

**Project Supervisor:** Gabriel Castrillo

**School:** Biosciences

**Description:** This PhD program will investigate new molecular mechanisms of seed/seedling-microbe interactions necessary to complete normal root development. This will allow us to add new regulatory components to root development pathways and better understand this process when the microbiota is present as it occurs in nature. Specifically, in the rotation the student will be working on activities related to the first year of the PhD project. Specifically, in the identification of which bacteria can produce active compounds which affect early root meristematic and patterning activity? To address this the student will screen 50 genome-sequenced bacteria strains isolated from Arabidopsis roots. This collection will be grown under different stresses commonly found in nature. These conditions will favour the release of specific bacterial-derived compounds into the medium. These compounds will be used to treat seeds of the Arabidopsis CYCB1;1:CYCB1;1-GFP line, that expresses the mitotic cyclin CYCB1;1, marker of cell division, to evaluate their effect on seed germination, early root development, and cell division. At the end of this rotation the student will identify bacterial isolates able to produce metabolites important for plant embryo development and become familiar with microbes and plant manipulation, confocal microscopy, phenotypes quantification, and statistical analysis.

**Location:** Sutton Bonington Campus;

**Full project description:** In humans, recent evidence favours the development of the foetus in a "sterile womb", exposed only to critical microbially derived compounds to complete its development. Unfortunately, in plants, it is completely unknown whether microbiota-derived compounds are relevant to early embryonic development. Fundamentally, it is known that, regardless of the plant species, seeds harbour up to 100 times fewer microbial species compared to other plant compartments. This reduced microbial diversity in seeds leads us to hypothesize that microbiota-derived metabolites, rather than direct interaction with the microbiota, may be required for normal embryo development.

This PhD program will investigate new molecular mechanisms of seed/seedling-microbe interactions necessary to complete normal root development. This will allow us to add new regulatory components to root development pathways and better understand this process when the microbiota is present as it occurs in nature. Specifically, the student

will study in year 1: Which bacteria can produce active compounds which affect early root meristematic and patterning activity? To address this the student will use 185 genome-sequenced bacteria strains isolated from Arabidopsis roots. This collection will be grown under different stresses commonly found in nature. These conditions will favour the release of specific bacterial-derived compounds into the medium. These compounds will be used to treat seeds of the Arabidopsis CYCB1;1:CYCB1;1-GFP line, that expresses the mitotic cyclin CYCB1;1, marker of cell division, to evaluate their effect on seed germination, early root development, and cell division. At the end of this year the student will identify bacterial isolates able to produce metabolites important for plant embryo development.

In year 2, we will identify what is the nature of these compounds? To identify the bacterial exometabolites, the student will analyse the metabolic composition of all selected bacterial supernatants in year 1, using liquid chromatography-mass spectrometry UHPLC-MS. Significant features obtained will be identified by matching the observed accurate mass of each compound with the m/z values available on the METLIN, KEGG, lipid MAPS and HMDB databases with the CEU Mass Mediator tool.

In year 3, we will investigate which bacterial genes are involved in the metabolism of these compounds? Since the bacteria used in this proposal are fully sequenced it is possible to identify bacterial biosynthetic genes clusters involved in the synthesis of the compounds characterised in year 2 using bioinformatic tools. We will validate whether the identify genes are involved in the compound synthesis by checking the effect of the mutant supernatants on the development of the seed/seedling. We predict that bacterial mutants lacking the capacity to synthesise these compounds will have no effect on the early meristematic activity.

Finally, in year 4, we will define how does this network of compounds optimize early root development to cope with environmental stresses? We will combine single cell RNAseq with plant mutants and overexpressing lines to identify and validate the regulatory network controlling the response of the root to the bacterial exometabolites. This knowledge will allow us to design combinations of compounds able to induce controlled changes in the root to maximise its functions.

**Full project location:** Sutton Bonington Campus;

[Manipulating targeted genes to improve barley resilience: characterising novel environmental stress regulators.](#)

**Project Supervisor:** Guillermina Mendiondo

**School:** Biosciences

**Description:** During the rotation the student will be received general training in molecular biology techniques for example: growing plants under control and stress conditions, root sampling, root anatomy using vibratome sectioning and laser ablation tomography, confocal microscopy to image sections and image quantification using rootscan or automated tools based on machine learning algorithms, crops bioinformatics, CRISPR, western Blot, barley transformation (tissue culture) apply to Crops. All these techniques will be a snapshot of techniques used in the main project.

**Location:** Sutton Bonington Campus;

**Full project description:** Impact of changing climatic conditions on global crop production. We need to increase the population to feed the world but while reducing the

reliance on fertiliser application. One solution is to develop varieties that have improved soil resource capture and thus improved yield under suboptimal conditions. Crop yields are globally affected by abiotic stresses such as drought, salinity and waterlogging. Ubiquitin-mediated proteolysis via the Plant Cysteine Oxidase (PCO) branch of the PRT6 N-Degron pathway controls the plant response by regulating the turnover of proteins involved in sensing and/or conferring tolerance to abiotic stresses. Root cortical senescence (RCS). Importance of cortical senescence towards this especially improving yield while reducing metabolic burden of maintaining cortical tissues under abiotic stresses. RCS is a very important breeding target for edaphic stress tolerance. The fundamental understanding about genes and mechanisms controlling this process is missing. Interestingly, we recently observed that mutants in PRT6 N-degron pathway have increased RCS. This project focuses on newly discovered and uncharacterized N-Degron pathway substrates to shed light on their specific downstream stress regulation functions, offering new opportunities for stress resilience research. We have previously identified two such substrates, BERF1 and RAF; this project will study their specific roles as key regulators of environmental stress. A recent paper showed RAF and BERF1 were upregulated in a transcriptional analysis, which indicated their important role of these gene in the development of root cortical senescence (RCS). Thus, in this DTP project we aim to dissect this to improve our fundamental understanding about genes controlling this process and identify novel genetic components/solutions that could help us develop varieties tolerant to abiotic stresses.

Work plan:

Year 1: Map the development of root cortical senescence during drought in the mutant and WT. Decide zones and timepoints for sampling roots and perform RNAseq of mutant vs WT under control and drought stress and sample different zones, including RCS formation to understand genes expressed. Prioritise some candidates' genes based on previous work and its relevance in drought, etc.

Year 2-3: Develop CRISPR, RNAi, reporter lines to validate downstream targets of PRT6 N-degron pathway functioning in regulating root cortical senescence.

Year 3-4. Perform drought experiment in soil columns in Hounsfield facility. Harvest roots for Laser ablation tomography and see whether RCS formation is impaired in the mutant and its impact on the cost benefit of forming RCS in the mutant vs WT. Also study shoot physiological parameters using PhenoSpex trait finder in the Hounsfield facility.

Year 4: Deploy novel genetic solutions to breeding companies.

Project output and impact: This project focuses on newly discovered and uncharacterized N-Degron pathway substrates to shed light on their specific downstream stress regulation functions, offering new opportunities for stress resilience research. This project therefore combines fundamental research into understanding the drivers for abiotic stress resilience, with application to generate resilient barley for deployment.

**Full project location:** Sutton Bonington Campus;

## Development and assessment of a novel sensitive method for the genetic analysis of complex traits

**Project Supervisor:** Cyril Rauch

**School:** Veterinary Medicine and Science

**Description:** The rotation will be tailored to the student's academic background. It will use genotypic and phenotypic data obtained from a BBSRC-funded project concerning the genetic basis for physiological responses to deficiencies in one-carbon (1C) metabolism (e.g., folate and vitamin B12 deficiency) in a sheep model.

- a. If the student has little experience in quantitative genetics, the period will be divided in two parts. Part I (5 weeks), the student will be trained in the field of quantitative genetics to perform genome-wide analysis using current/classic methods (i.e., Genome Wide Association studies; GWAS) and our new method (Genomic Informational Field Theory; GIFT). Part II (4 weeks), the student will undertake a pilot study to compare the power of each method for genetic association using two 1C metabolites (e.g., folate and B12).
- b. If the student is already familiar with current quantitative genetic methods (i.e., GWAS), the 9 weeks will be undertaken as a single block during which the student will be trained to perform genome-wide analysis using GIFT and to compare this to GWAS for four metabolites (e.g., folate, B12, choline and methionine). There would also be follow-up analyses to predict the functional significance of genetic associations.

**Location:** Sutton Bonington Campus;

**Full project description:** Background: Genome-wide association studies (GWAS) have facilitated the understanding of many important biological processes. The issue however is that GWAS require large amounts of data to identify significant associations when complex traits are involved. Over the past 2 years the team of supervisors has worked on developing a new method (Genomic Informational Theory: GIFT), specifically designed to determine genotype-phenotype associations in cases where the sample size (i.e., number of human or animals involved) is small. The main conceptual change brought about by the new method is the way information previously considered to be 'noise' (i.e., unexplained variation in the data) is included in the model. The new method integrates the 'noise' into the model by analysing the mixing of genotypic states on a single scale defined by the phenotypic values ranked as a function of their magnitude. This new method contrasts with classic GWAS methods which consider each genotypic state on a separate phenotypic scale, with the link between genotype and phenotype defined simply by the differences between phenotypic means. Our new method has unprecedented potential to detect small genes effects even in relatively small sample sizes.

**Project:**

Interindividual variability in epigenetic gene regulation in humans and sheep can arise as a consequence of single-nucleotide polymorphisms (SNPs) within genes involved in regulating one-carbon (1C) metabolism, associated epigenetic regulators, and differentially methylated target DNA sequences. The project will use GIFT and GWAS to identify functionally significant SNPs in 1C metabolic genes and associated epigenetic regulators. To do so, we have recently genotyped around 360 sheep of a single breed to study the genetic regulation of 1C metabolism and the inter-individual variability in metabolic responses to micronutrient deficiencies. The study made use of a custom array with 3,129 SNP probes for variants directly involved in the regulation of intermediary 1C

metabolites and chromatin modifiers. The objectives this DTP are to undertake association analyses to identify functionally significant genetic variants and to compare the efficiencies of GWAS and GIFT. This will extend the analyses undertaken during the rotation to include all 1C metabolites and, through enrichment analyses, to determine which method can best identify functionally significant SNPs.

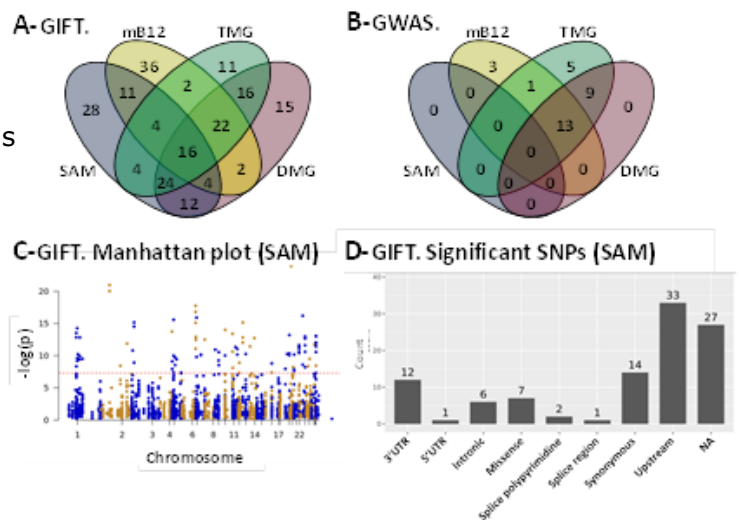
Work plan:

Phase-1: Advanced training with GIFT such as to explain its conceptual difference with classic GWAS. Will allow the student to redefine key notions from genetic used by GWAS within the framework of GIFT.

Phase-2: Will involve generating simulated data enabling the student to compare GIFT and GWAS methods under different scenarios linked to variation in genotypes and/or phenotypes.

Phase-3: Will involve running GIFT and GWAS to identify functionally significant genetic variants in 1C metabolism and directly associated metabolic genes and epigenetic regulators.

Phase-4: Will extend these analyses to include three further contrasting breeds native to the UK, and will assess the extent to which variant allele frequencies differ between these four breeds (equivalent to 'ethnic human populations'); the hypothesis being that some breeds (ethnic groups) may be genetically more susceptible to dietary deficiencies in 1C metabolite such as folate and B12.



**Full project location:** Sutton Bonington Campus;

[Investigating the mechanisms of pH homeostasis linked temperature stress response in plants](#)

**Project Supervisor:** Dr. Rahul Bhosale

**School:** Biosciences

**Description:** Maintaining pH homeostasis is critical for optimal plant growth and development, and resilience to environmental stress. Fluctuations in pH levels of plant cell or its environment can significantly impact protein and enzyme activity, water and nutrient capture and use efficiency, and the management of harmful reactive oxygen species (ROS). Therefore, it's essential to study mechanisms regulating pH homeostasis in plants under stress conditions to develop resilient plant varieties.

During this lab rotation, the student will validate the relevance of a key pH homeostasis gene, which exhibit differential regulation in roots under high-temperature conditions compared to control conditions.



- In week 1-2, the student will grow wildtype and homozygous T-DNA mutants of identified genes under varying temperature conditions.
- Weeks 3-4 will involve quantifying root growth and architectural parameters during stress and recovery phases, as well as sampling root tips for further analysis.
- Weeks 5-8 will be dedicated in performing pH measurements in cellular compartments and quantifying ROS levels in the root tips and conducting confocal microscopy to assess root anatomical parameters.
- Finally, in the 9th week, the student will review the literature and write a report assessing the impact of temperature stress has apoplastic pH, ROS and root anatomy in the mutants versus wildtype plants.

**Location:** Sutton Bonington Campus;

**Full project description:** Background: Increasing global temperatures from climate change stress plants, altering their anatomy, physiology, and biochemistry, impacting growth and productivity. Understanding these adaptations is crucial for developing heat-resistant crops. While heat stress effects on shoots are well-studied, root responses remain unexplored.

Our latest research identified a heat-induced molecular mechanism controlling root tip growth. Apoplastic pH changes in root growth zones after heat stress, followed by ROS level and distribution changes, drive adaptations in root growth (Lale et al., Unpublished). Yet, how the apoplastic pH responds to temperature stress and how it regulates downstream machinery in finetuning root growth during heat stress remains unclear.

To address this, we've found a pH homeostasis gene family member, PHH, regulated during heat stress. Its mutant shows impaired ROS and anatomical changes. A DTP student will determine how exactly heat stress regulates PHH expression in the root tip and how it controls downstream processes.

Work plan:

Year 1-2: Determine where PHH functions in the root. To study the expression of PHH in roots, the student will create a transcriptional and translational reporter line in the Col-0 background using a viral 2A peptide method. They will then analyse the reporter in control and temperature stress conditions to identify where the gene/protein is expressed. Finally, they will use Golden Gate cloning to reintroduce the gene into the mutant background with its native promoters from the expressed tissues/zones to rescue the pH-ROS-anatomy mechanism defect, all via the Agrobacterium-mediated floral dip method.

Year 2-3: What regulates PHH expression during heat stress. In silico analysis of the PHH gene's promoter revealed 21 potential TF regulators. However, only 6 TFs showed differential expression under heat stress, with two of them exhibiting similar regulation as PHH. These two TFs are Heat Shock Factors (HSFs), known to control gene expression during heat stress. The student will identify mutants for both HSFs and assess PHH expression during heat stress using qPCR. Positive results will lead to crosses with the phh mutant for pathway validation. If confirmed, a biochemistry experiment will demonstrate that these HSFs directly bind to the promoter to regulate its expression.

Year 3-4: What are the downstream components of PHH. The student will conduct RNA sequencing experiments on both the PHH mutant and WT (wild type) plants under varying time points of heat stress. This analysis aims to pinpoint key genes responsible for differential regulation associated with ROS (reactive oxygen species) and root growth

regulation. Finally, student will construct a detailed molecular mechanism model that works upstream of ROS-Anatomy processes controlling root growth.

Output and impact: The key molecular components that are responsible for regulating root growth under heat stress. This research has the potential to open up new avenues for developing or identifying crop varieties that can withstand the rising soil temperatures driven by climate change.

**Full project location:** Sutton Bonington Campus;

### The identification of novel methods for re-emerging Enterococci pathogen management in intensive poultry farming

**Project Supervisor:** Adam Blanchard

**School:** Veterinary Medicine and Science

**Description:** During the lab rotation the student will get accustomed to traditional microbiology techniques, including isolation, culturing and characterisation of the enterococcal species and phage. The will also isolate novel phage and learn how to test for infectivity. The student will also learn bioinformatic skills, learning core unix and how to run more complex workflows in command line.

**Location:** Sutton Bonington Campus;

**Full project description:** Enterococci are a group of bacteria that are both commensal and opportunistic pathogens in humans and animals. Some strains of enterococci exhibit probiotic properties, promoting gut health and aiding digestion by improved nutrient absorption, enhance the immune system, and the reduction of harmful pathogens in the gastrointestinal tract through competitive exclusion. However, there are major concerns associated with some enterococcal species being labelled as re-emerging pathogens, potentially due to the reduction of antibiotics as growth promoters and for disease prevention. Cases of enterococcus associated disease in the poultry industry have increased from 0.4% in 2006 to 12.9% in 2020. As poultry production intensifies to meet the growing demand for protein, understanding and managing enterococci becomes crucial for maintaining bird health, food safety, and environmental sustainability. This poses a new threat, due to *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus cecorum*, being linked to an increase in locomotor disorders and septicemia and endocarditis in broiler chickens.

The poultry industry, like many other livestock sectors, faces the challenge of decreasing the use of antimicrobials to limit antibiotic resistance. Enterococci are notorious for their ability to develop resistance to multiple antibiotics, including those commonly used in human and veterinary medicine. Finding alternative strategies to manage bacterial infections in poultry is crucial. A new approach to flock health is with alternative growth promoters. Poultry producers can explore alternative growth-promoting strategies such as prebiotics, probiotics, organic acids, and phage therapy. These natural additives can enhance feed efficiency and bird performance while reducing the incidence of antibiotic resistance. Research on phage therapy and other biocontrol methods targeting pathogenic enterococci presents a promising avenue for reducing the reliance on antibiotics.

The project will be hosted by Dr Blanchard the Streptococcal Research Group (SRG) in the School of Veterinary Medicine and Science, University of Nottingham in collaboration with the Dr Alkhtib at the Poultry Research Unit, Nottingham Trent University. The

project will start with the curation and characterisation of a collection of enterococci isolated from poultry houses, overseen by Dr Clarke MRCVS, and collected through partner diagnostic laboratories. A library of contemporaneous phage will then be isolated and screened against multiple enterococci, using high throughput methods, to determine those which can infect and effectively lyse the target pathogenic species, without interfering with commensal species. Using novel machine learning algorithms, developed by Dr Atterbury, putative phage will be isolated and cultured for further testing using bacterial impedance cytometry to select and optimise targeted candidate therapeutic phage.

**Full project location:** Sutton Bonington Campus; Brackenhurst Campus;

[How mechanosensing drives root penetration in hard soil?](#)

**Project Supervisor:** Bipin Pandey

**School:** Biosciences

**Description:** During the nine-week rotation, the student will evaluate the root architecture and compaction responses of the mechanodeficient (md1) rice mutant using non-invasive micro-Computed Tomography ( $\mu$  CT) imaging. Equally germinated seedlings of the md1 mutant and WT seedlings will be grown in noncompacted and compacted soil conditions for one and two weeks in 20 cm 30 cm deep soil columns. Student will be trained to use and quantify maximum rooting depth, root hull volume, root thickness, branching and total root length. To investigate the changes in cell shape and volume, student will perform confocal imaging of cleared root tips harvested from noncompacted and compacted soil.

In summary, we envision that these interdisciplinary approaches encompassing the non-invasive imaging of md1 roots in compacted and noncompacted soil, root anatomical imaging with confocal microscopy will provide a comprehensive insight into the how root tip sense mechanical environment, respond and modify the cell shape, function and helps to navigate effectively in compacted soil conditions.

**Location:** Sutton Bonington Campus;

**Full project description:** Soil compaction is major modern agronomic problem leading to substantial global yield losses. Over the recent past, the sizes of farming machinery have increased about 10 times which compresses the topsoil as well as the subsoil making roots to extremely challenging to forage for critical resources like nutrient and water. While it was conventionally believed that roots were unable to penetrate compacted soil due to its excessive hardness, a ground-breaking discovery our team has elucidated that plant roots actively choose not to penetrate hardened soil due to the accumulation of ethylene within microscale soil pores. (Pandey et al., 2021, Science).

Despite this breakthrough discovery of how roots sense soil compaction, the molecular mechanism underpinning how plant roots respond to compaction is not known. In this BBSRC DTP project, we aim to discover the root adaptive response mechanism in compacted soil conditions. Specifically, we aim to reveal how compaction regulates the biophysical, structural, and functional traits of cell walls, which provide the structural and functional framework to receive and respond to physical forces such as compaction. During the four year PhD programme, the student will be trained, supervised to achieve following objectives.

Map mechanical properties of rice root cell walls in compacted soil. Perform state-of-the-art phonon imaging to assess the mechanical and viscoelastic properties of epidermal, exodermal, cortical and endodermal cell wall in Wild Type rice root tips subjected to soil compaction treatments. Subsequently, image and analyse cell wall stiffness of rice root tips grown in compacted soil.

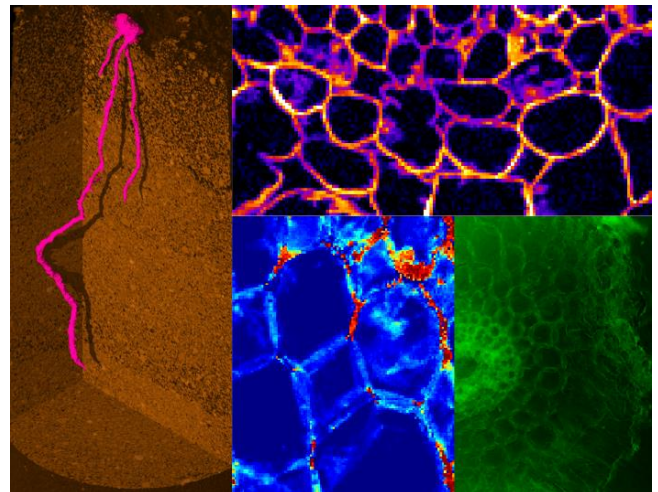
Generate compaction responsive reporters and mechanoproofing rice roots to monitor soil compaction stress: Generate cell specific CRISPR mutants, overexpressing and transcriptional reporter lines of the genes related cell wall remodelling, mined from recently generated single cell transcriptomics data of rice root grown in compacted soil. This objective will focus on discovery of novel compaction responsive cell wall stiffness regulating genes curated from the already generated single cell transcriptomics data of the rice root tips (host lab) grown in compacted (1.6 g/cm<sup>-3</sup>) and noncompacted soils (1.2 g/cm<sup>-3</sup>). Finally, top 2 candidates will be genetically engineered using CRISPR editing, transcriptional fusion with VENUS and overexpressing approaches to assess their impact on root cell wall composition, rigidity and mechano-response in compacted soil conditions.

Imaging compaction responses of cell wall mutants in compacted soil

The student will evaluate the compaction performance of mechanodeficient rice lines using computed tomography (CT) imaging, Brillouin microscopy, and anatomical studies in noncompacted and compacted soil conditions.

To summarize, this innovative BBSRC DTP proposal will generate valuable outcome and new knowledge that how soil compaction forces cell wall to change their structure, dynamics, morphology, growth, and mechanical properties to regulate root growth in compacted soil conditions. Overall, this interdisciplinary project goes above and beyond current state of the art and pioneer integration of photonics, non-invasive CT imaging, advanced single cell transcriptomics and compaction reporter to reveal the underlying design principles of how soil compaction remodel cell wall to change cell shape and growth.

**Full project location:** University Park; Sutton Bonington Campus;



[Smart adsorbent materials to mitigate antimicrobial resistance in dairy farm wastewaters \(SAM-FARM\)](#)

**Project Supervisor:** Rachel Louise Gomes

**School:** Engineering

**Description:** This novel interdisciplinary PhD project involves developing adsorbent materials to treat dairy waste/waters for the reduction of antimicrobial resistance (AMR) and recovery of metal and antibiotic pollutants. The selected student will have an exciting opportunity to work across the Faculty of Engineering and School of Veterinary Medicine & Science and visit the University of Nottingham Dairy Farm to introduce them to the global challenge and University research taking place on antimicrobial resistance in the environment. Uniquely, this will also include materials synthesis and analytical

approaches that look at mitigating AMR, as well as sustainability and waste to wealth that will be implemented in the linked PhD project.

In order to develop effective novel sorbent materials for removal metal and antibiotic pollutants that drive AMR, it is important to identify and quantify the pollutants (biological and chemical) present in dairy wastewaters and to understand their impact on the sorption process and interactions with resistant microorganisms and genes (ARB/ARG). To this end, the student will participate in collecting (at the University Dairy Farm) and characterising samples from dairy waste/waters. The microbial content will be assessed in the microbiology labs where the student will learn techniques used for cell culture, growth and viability. Understanding how effective we are at mitigating these AMR-driving pollutants can unlock huge opportunity to both reduce/remove AMR in the dairy farm environment and turn pollutants into products (supporting circular economy thinking).

**Location:** University Park;

**Full project description:** Project Aim: This project will develop unique sorbent materials to treat dairy waste/waters for the reduction of AMR and recovery of metal and antibiotic pollutants. Removal of pollutants these wastes will enable safe re-use as a fertiliser for crops without reduced AMR.

Many farms, including the University of Nottingham Dairy Farm dispose of tonnes of metal-containing cattle footbath waste (used to protect against lameness caused by bacteria) into slurry wastewaters per annum. This is a potential source of environmental metal pollution and antimicrobial resistance (AMR), with these wastewaters subsequently spread as fertiliser onto crops. The wastewaters also contains antibiotic-polluted milk, used water for washing farm equipment, to waste from the dairy herd. Both metal ions and antibiotics are co-selection drivers for antimicrobial-resistant bacteria (ARB).<sup>1</sup>

Understanding how AMR develops is important, and will inform on part of this PhD project. However, to remove pollutants that drive AMR becomes a win-win for both mitigating AMR and managing pollution in the environment. To achieve this, we can use metal-organic frameworks (MOFs) which are sorbent materials that show great promise for removing of metals and antibiotic drugs from waste/waters.<sup>2</sup> The MOF sorbent materials have certain features including high porosity and tuneable functionality that provides the opportunity for developing highly selective MOFs for sorption of metal and antibiotic pollutants from waste/waters.<sup>2</sup> From a cost-benefit perspective, electromagnetic (EM) energy offers two advantages; it has been used for 'curing' plasmid DNA<sup>3</sup> and can be used to improve sorption through heating<sup>4</sup> the waste.

The project will involve identifying and quantifying the species present in real-world dairy waste samples. It is important to understand what is present in the waste and in what concentrations as this will strongly impact the sorption process. A range of novel MOFs will be prepared rapidly and efficiently using bespoke microwave technology, with no prior knowledge required and the rotation project will introduce and provide training on how to make MOFs. The stability of these MOFs will be assessed under temperature and pH conditions relevant to dairy waste/waters. Next, variables which affect the sorption kinetics (ratio of MOF to metal ion and/or antibiotic, concentration, pH, competitive sorption, the impact of microwave heating, MOF functionalisation and pore size) will be assessed using environmental matrices which mimic the conditions of the dairy waste. Results will enable us to develop MOFs with high sorption capacity and selectivity and to optimise removal of pollutants. The effect of microwave heating on cell lysis and/or plasmid curing will be investigated. The final established process (novel developed MOFs and microwave conditions) will then be tested on real-world dairy farm wastewaters

Alongside we will evaluate the antimicrobial resistant organisms to understand how effective our technology is at removing the pollutants driving AMR.

This exciting trans/interdisciplinary PhD project offers the unique opportunity to prepare novel materials for AMR mitigation whilst developing understanding and expertise of how only interdisciplinary skills can address the global challenge of AMR in the environment. The project addresses concerns in food security, food safety, and AMR with the potential to deliver economic and societal impact in UK and global agriculture.

References:

1. BMC Genomics, 2015, 16:964; J. Med. Microbiol., 2015, 64:471-97.
2. Chem. Eng. J., 2017, 322:366-374; ACS Appl. Mater. Interfaces, 2016, 8, 30294-30303.
3. Arch. Microbiol., 2013, 195:181-188.
4. Bioresource Technol., 2014, 160:3-14.

**Full project location:** University Park; University of Nottingham, either in Faculty of Engineering, University Park Campus or School of Veterinary Medicine & Science; dependent on interests and expertise of the PhD student.



How a gaseous hormone is made, mobilized and mediates stress responses in plant tissues.

**Project Supervisor:** John King

**School:** Mathematics

**Description:** The rotation will comprise confocal imaging of (i) ACO-GFP (transcriptional) reporters, to observe dose-dependent ethylene production in different cell types of Arabidopsis root tips, helping both to reveal the ethylene responsive nature of ACO genes and to discover which of the five ACO genes play prominent roles in ethylene biosynthesis; (ii) ethylene reporter EIN3-GFP/ein3eil1, with different doses of ethylene at different time points, helping establish how fast ethylene accumulation responds to external ethylene level. These observations will inform model parametrisation to understand how and where ethylene is synthesised and moves within or across the cells. Thus as part of the rotation the student will be exposed to a number of routine and some specialised techniques in Molecular Cell Biology, plant phenotyping and plant physiology such as:

- Reporter gene studies
- In situ immunolocalisation
- Confocal Microscopy
- Lightsheet Microscopy
- In vitro plant culture

- Microtome sectioning
- PCR and RT-PCR
- Image processing packages including ImageJ and WinRHIZO.

This experience will be of considerable value to a mathematically trained student.

**Location:** Sutton Bonington Campus;

**Full project description:** Ethylene is a vital gaseous signal regulating critical plant developmental and stress responses. A recent publication by the co-host lab reported roots use ethylene to sense soil compaction [Pandey et al, 2021, Science]. They discovered diffusion of ethylene released by roots is suppressed in compacted soils as the proportion of inter-connected air spaces are significantly reduced. In compacted soils, high ethylene concentrations build up around and in root tip tissues, triggering growth inhibition. However, after genetically disrupting the ethylene response machinery, mutant roots were able to penetrate compacted soil (unlike the wildtype). Breeding crops resilient to soil compaction stress is vital for global food security efforts. The co-hosts recent work revealing a role for ethylene provides an exciting means to attain this. However, to facilitate this goal, fundamental knowledge about how ethylene is made, mobilized and mediates compaction stress responses in plant roots is required.

The DTP project will exploit a mathematical-modelling-based systems biology approach to determine how ethylene moves between its sub-cellular site(s) of synthesis and is eventually release from root tissues into the soil. The student will initially adapt an anatomically accurate vertex model of the Arabidopsis root that captures key sub-cellular, cellular and tissue scale features of the root tip site of ethylene synthesis. This hydrophobic gaseous molecule favours diffusion across ER networks (via plasmodesmata (PD) connecting one cell to another) rather than hydrophilic cytosolic and apoplastic routes. To reveal the pathway of ethylene movement at a subcellular scale, the student will exploit an ethylene biosensor to reveal if the signal is moving between and within cells via PD and ER, respectively. Based on the distance, membranes, and other compartments that ethylene needs to traverse, the student will be able to precisely model, with the help of lead supervisor John King and collaborator Olivier Martin (Paris), how ethylene reaches the root surface. Collectively, these innovative systems biology approaches promise to unveil entirely new routes of ethylene movement across root tissue types and bridging from subcellular to root-rhizosphere scales.

The DTP will involve cutting edge imaging technologies such as Light Sheet microscopy and hormone biosensors in addition to mathematical modelling and plant cell biology. This represents an ambitious, novel, yet realistic project as it relies on well-established techniques and state-of-the-art equipment in the co-host lab. The main supervisor's mathematical modelling expertise and knowledge of the topic area will ensure that the project produces high-quality results and that the student will receive exemplary guidance as they embrace new techniques in a highly interdisciplinary environment.

**Full project location:** University Park;

## Characterisation and validation of Cougar-derived candidate genes for resistance to *Septoria tritici* blotch (STB) in wheat

**Project Supervisor:** Rumiana Ray

**School:** Biosciences

**Description:** This rotation project will be both lab and office based. The student will use existing NILs from the wheat breeder which will be exposed to *Zymoseptoria tritici* causing *Septoria tritici* blotch (STB) on wheat to confirm their physiological and disease phenotypes with virulent and avirulent isolates of the pathogen. The student will learn microbiology, microscopy, and plant pathology techniques, to perform inoculation bioassays and carry out physiology and disease phenotyping. He or she will develop biostatistical skills to analyse their data and will learn how to carry out bioinformatics analysis using specialised software to identify candidate genes for resistance and annotate them. The student will carry RT PCR for key molecular markers involved in plant defence. In addition as part of the rotation the student will be exposed to a number of routine and some specialised techniques such as Image processing packages including ImageJ and using Licor 6400 gas exchange analyser for photosynthesis and fluorescence measurements.

**Location:** Sutton Bonington Campus; NIAB-EMR;

**Full project description:** *Septoria Tritici* Blotch (STB) caused by *Zymoseptoria tritici* is considered economically the most damaging disease of wheat, associated with yield losses of up to 50% in years of severe epidemics in UK and Europe. The pathogen is capable of rapidly evolving and developing resistance to most currently available commercial fungicides and therefore chemical control is not a sustainable option for protection against the disease. The most effective control is likely to be derived via varietal resistance or tolerance which requires improved understanding of the regulatory mechanisms of defence against the pathogen and the identification of novel physiological traits and genes associated with disease resistance.

Plants have evolved photoprotective mechanisms to avoid and repair damage to the photosynthetic apparatus due to environmental stress. The same mechanisms are also involved in the regulation of plant defence against pathogens via redox signalling originating in the plant chloroplasts commonly assessed by non-photochemical quenching (NPQ) of chlorophyll fluorescence. Recent work by the University of Nottingham (Plant Path 70:1421-1435) using susceptible and resistant wheat genotypes to *Z. tritici* identified that photoprotective responses of the resistant cultivar Cougar were significantly affected by the pathogen during disease development and NPQ was the key marker for disease resistance response.

We have Near Isogenic Lines (NILs) from the breeding company RAGT which have been phenotyped using microscopy, disease, biochemical and physiological assays to determine which traits coincided with the major QTL for resistance to STB from the cultivar Cougar. Selected lines were taken through further transcriptomic and molecular analysis to identify candidate genes and regulators of photoprotective processes involved in disease and defence. Using the latest IWGSC assembly of wheat genome, the student will annotate and confirm the candidate genes from these data sets, hypothesizing which of them are involved in the resistance. The student will test the above hypothesis by assessing the function of selected candidate genes using Virus-induced gene silencing (VIGS) approach, that is well established in the laboratory of Dr Kostya Kanyuka (Plant Physiol 160: 582-90). Mechanisms of resistance conferred by the candidate genes will be investigated using molecular biology approach to determine how photoprotective and defence pathways are regulated.



The aim of this study will therefore be to validate candidate genes for resistance from an important source of UK STB resistance and confirm the physiological and molecular mechanism behind it. If a strong candidate is identified, attempt at cloning of the gene will be made.

The objectives (milestones) of the PhD project will be to:

1. Annotate and confirm candidate genes for resistance and photoprotection from recent transcriptomics and other biological data sets from NILs of an important UK STB resistance from RAGT.
2. Investigate SNPs in the region of interest using available genome assemblies.
3. Test candidate gene functionality using VIGs approach.
4. Investigate the mechanisms of resistance and compatibility with avirulent and virulent isolates of *Zymoseptoria tritici*.

**Full project location:** Sutton Bonington Campus; NIAB-EMR;

[Enhancing heat tolerance in wheat from wheat wild relatives to improve food security.](#)

**Project Supervisor:** Dr Stella Edwards

**School:** Biosciences

**Description:** The rotation will focus primarily on screening homozygous introgression lines made from crosses of wheat with wheat wild-relatives from the Nottingham Wheat Research Centre (WRC). Some wheat wild-relatives are known for heat tolerance which is a vital trait required to reduce wheat yields in warming climates. Comprehensive interdisciplinary training given at the WRC in plant genetics, molecular biology, cytogenetics, plant physiology and photosynthesis.

- Weeks 1-2: A sub-set of potentially heat tolerant lines will be grown in the WRC glasshouses and growth rooms. Wheat husbandry of how to grow and distinguish wheat growth stages will be learnt for reproducible measurements. Molecular analysis using KASP genotyping (DNA extraction, PCR) will be determined to characterise the genotypic basis of the lines screened.
- Weeks 3-6: Training in plant physiology will be given using a high throughput screen to analyse heat tolerant traits. Infra-red gas analysis used to measure photosynthetic heat tolerance along with complementary physiology techniques.
- Weeks 6-9: R programming language taught to analyse data. Lines of interest will be further targeted.

Further to the rotation, lines of interest may enter a breeding programme (where wide-crossing techniques, KASP genotyping, cytogenetic analysis (GISH) would fully characterise heat tolerant lines to benefit food security.

**Location:** Sutton Bonington Campus;

**Full project description:** Background: It is acknowledged that crops such as wheat which feed over 2.5 billion people world-wide contributing 20-50% of daily calorie intake and 20% of protein consumption in wheat-growing areas, has limited natural genetic diversity. This stemmed from a genetic selection pressure during domestication. This lack of genetic variation is expected to present significant challenges in the future, particularly in enhancing biotic and abiotic stress tolerances and improving key traits such as photosynthesis, which is highly susceptible to damage under the heat stress conditions associated with climate change. With wheat production set to decline by 6%

for every 1°C rise in temperature, the need to maintain yield production under global warming is vital.

Wheat wild relatives have retained their genetic diversity as they were never subjected to domestication, which suggests that they still represent a significant source of untapped genetic variation for many, if not all agronomic traits. This variation can therefore be transferred into wheat by crossing, the goal of which is to produce introgression lines containing these beneficial alleles from the wild relatives. This is the current objective of the BBSRC Nottingham Wheat Research Centre (WRC).

Wheat wild relative species from the *Triticum* family serve as donors of genetic material to enhance wheat growth and yields which are limited by abiotic factors. These *Triticum* species offer a key source of variation in traits for improved heat tolerance (one of the significant abiotic factors affecting wheat yields) a trait that wheat has either lost or never obtained.

At the WRC, a pre-breeding program has created more than 200 homozygous introgression lines of wheat containing genetic diversity for many agronomic traits of interest from *Triticum urartu* and *Triticum timopheevii* wheat wild relatives. It has previously been shown at the WRC that there is a vast array of genetic variation in the wheat wild relatives for photosynthetic traits and in some of the introgression lines produced. The opportunity to further screen over 200 homozygous introgression lines generated through crossing using high throughput screening methods available at UoN can lead to promising further genetic variation in photosynthetic traits which can be utilised to boost food security under climate change.

Objectives Years 1 and 2. Physiological analysis. Screening of 200 homozygous *Triticum* wheat wild-relative introgression lines produced at the WRC for photosynthetic heat tolerant traits in collaboration with Professor Erik Murchie and the WRC. Techniques include (but not limited to) high throughput Fluorcam screening of chlorophyll fluorescence to detect heat tolerance. Infra-red gas analysis (Li-Cor-6800) photosynthetic measurements, and hyperspectral analysis in the WRC glasshouses and growth rooms.

Objectives Years 3 and 4. Molecular characterisation and pre-breeding of lines of interest. Lines of interest can be characterised in more detail for photosynthetic heat tolerance and developed through a pre-breeding programme to target genes of interest by producing smaller introgressions without deleterious genes using techniques such as wide-crossing, KASP marker genotyping and molecular cytogenetic analysis (GISH) with the aim to produce new wheat varieties which can withstand climate change.



**Full project location:** Sutton Bonington Campus;

## Paramyxovirus spillover between humans and livestock - potential for a new pandemic?

**Project Supervisor:** Alexander Tarr

**School:** Life Sciences

**Description:** The student will undertake a project interrogating the ability of paramyxoviruses to mediate cell entry in different species. Synthetic genes encoding an expression construct of the H gene (haemagglutinin, responsible for cell binding) and F gene (fusion protein, responsible for fusion of viral envelope and host cell membrane) of reference strains of both human parainfluenza virus 2 and bovine parainfluenzavirus 3 will be commercially synthesised, generated. These will be used to generate novel pseudotyped viruses using an established lentivirus-based expression system. The student will assess protein expression, virus particle formation and infectivity in a range of reference cell lines to determine the cell entry tropism of these two viruses. Specificity of entry will be determined using known chemical fusion inhibitors. The student will also learn about the genetic diversity of paramyxoviruses and perform bioinformatic analysis of existing virus sequence datasets to identify conservation between different virus species.

**Location:** QMC;

**Full project description:** The SARS-CoV-2 pandemic highlighted the potential for spillover of previously unknown viruses from wildlife reservoirs to animals and humans. It is unclear which other virus species have the potential to transmit between host species and pose a threat to both human and animal health, with implications for future food security. Paramyxoviruses are a large group of viruses with varying potential to cause pathogenesis. Some, such as the henipaviruses, Hendra (HeV) and Nipah (NiV) are known to have a very broad mammalian host range, causing disease in humans and domesticated animals often with fatal consequences. This impacts not only on human health, but also pose a significant health concern for livestock animals with associated cost in food security. Other paramyxoviruses have pathogenesis limited to respiratory disease (e.g. bovine parainfluenza virus 3), while furthermore are responsible for severe systematic disease (e.g. rinderpest virus). Host and cellular tropism is initially defined by the ability of viral attachment proteins to recognise cellular receptors (often variants of sialic acid molecules) expressed on the plasma membrane. However, little is known about the specific determinants of tropism on the viral attachment protein haemagglutinin that define the differences in ability of the virus to enter host cells. Understanding how variations in the molecular structure of the haemagglutinin and fusion proteins affect receptor binding and virus tropism will provide insight into the potential for different paramyxovirus species to transmit between different host species.

Laboratory research of paramyxoviruses is restricted by the highly pathogenic nature of some species, and the potential threat to human and animal health. As such, model infection systems are required to investigate aspects of cellular infection. We have developed advanced retrovirus-based pseudotyping models that have been applied to investigate the entry pathways of diverse virus species that would normally require high level biological containment facilities, including hepatitis C virus, hepatitis B virus, Ebolavirus, and rabies virus. In this project we will apply this expertise to investigations of the entry of paramyxovirus species and the assess the impact of genetic variation in virus attachment proteins on host tropism.

This project aims to interrogate the potential for cross-species transmission of paramyxoviruses, including parainfluenzaviruses, between humans and livestock animals. Initially, reference strains representing paramyxovirus genera Morbillivirus,

Respirovirus, Henipavirus and Rubulavirus will be synthesised and expression constructs cloned for the purposes of pseudotype generation. Pseudotype infection will be assessed in a range of reference cell lines to determine the permissiveness of cell lines to entry of the different viruses.

Amino acid variation in the haemagglutinin and fusion proteins of different, related species of paramyxoviruses will be assessed from publicly available sequence databases, and from NGS datasets previously generated in our laboratory. This variation will be modelled onto existing structures of these proteins, informing design of novel expression constructs that will be incorporated into retroviral pseudotypes. Cellular tropism will be determined for these variants.

Together, this project will identify determinants of cellular tropism of entry of paramyxoviruses, providing new tools for investigations of therapeutic intervention for infection in livestock and humans.

**Full project location:** QMC;

[One Health surveillance approaches to fight AMR using Artificial Intelligence and big data mining](#)

**Project Supervisor:** Tania Dottorini

**School:** Veterinary Medicine and Science

**Description:** The student will be joining an exciting multidisciplinary team based in Professor Dottorini's lab. Dr. Dottorini is a Professor in Bioinformatics at the University of Nottingham (UK and UNNC) and the Director of the Centre for Smart Food Research in the China Beacons Institute. She is currently the UK academic lead of several national and international research projects (MRC and BBSRC) dedicated to developing new AI-based solutions to study the emergence and spread of antimicrobial resistance in several ESKAPE pathogens.

The supervisory team, together with the strong international links (UNICEF, Bangladesh National Centre for Diarrhoea, University of Pretoria, Food and Agriculture Organization of the United Nations, CFSA China) offers a unique combination of expertise in machine learning, bioinformatics, sequencing, cloud computing, microbiology, infection control, post-genomic statistical and computational approaches, microbiology and biofilm formation. During the nine-week rotation, the student will receive training in cutting-edge bioinformatics, high-throughput sequencing and machine-learning techniques applied to study bacterial genomes and their capacity to acquire resistance. In addition, he/she will be trained on how these techniques can be applied to the field of microbiology.

**Location:** Sutton Bonington Campus;

**Full project description:** Spread of antimicrobial resistant microorganisms and AMR at the human-animal-environment and food interface is a major global concern. The transmission of AMR can take place through different routes and pathways, however, the most likely one is the food chain, either indirectly (food consumption) or directly (contaminated food/animal handling and/or manure/faecal contaminated environment contact). Each year worldwide food-borne diseases cause 600M cases and 420,000 deaths. *Salmonella enterica*, *Escherichia coli*, *Campylobacter coli/jejuni*, *Enterococcus faecium* and *Staphylococcus aureus* are among the major pathogens causing food borne diseases. The increasing observation of AMR across food-borne pathogens is a growing

concern both across Europe and worldwide. Despite this, AMR surveillance is poor outside clinical settings, and resistant bacteria may circulate undetected between humans, livestock and the environment.

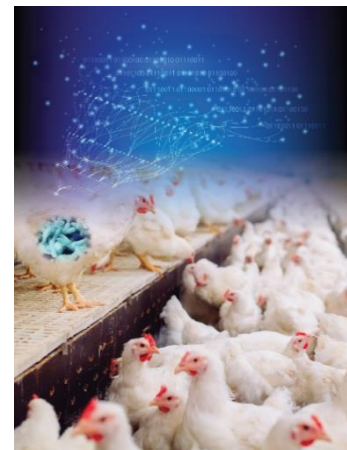
To fully understand the AMR issue and identify the most efficient points of control, we must have a 360-degree monitoring approach covering all relevant stages and reservoirs of the interconnected system of humans, animals, environment, and food. With the proliferation of collectable information, research has been gradually moving towards the adoption of the latest technologies in machine learning (ML) and big data mining to implement precision farming. Conventional surveillance methods and tools cannot cope with such complexity and scale of data, therefore more sophisticated methods are needed. Recent years have seen tremendous growth of machine learning and AI-enabled methods expanding the breadth of investigation. Key to this is the development of cost-effective surveillance capable of integrating different sources and scales of data and covering all sectors of the One Health approach. The project will utilise a large amount of heterogeneous data from farms, feed and livestock, including sequencing, microbiological and sensor data, collected as part several ongoing funded grants. The project methodology will focus on data mining and machine learning approaches, and there will be the opportunity to be involved in NGS sequencing of samples and experimental work.

The aim of this project is to introduce novel ML approaches to precision farming, based on a better understanding of infection, resistance, mobility and relationships between different pathogens within these environments. This will be achieved through identifying biomarkers resistance development, exposing external correlations, and experimentally validation.

WP1: Use infection statuses, resistance profiles and mobility markers as detected from processing of biological samples to tag all the other data collected contextually from environment, livestock, water and feed.

WP2: Using the statistical, data mining and machine learning methods and developing new pipelines to identify biomarkers of pathogens and uncover correlations with pathogen modifications observed via biological sampling.

WP3: Conduct experimental validation of identified biomarkers/correlations using gene knock out/knock in approaches and other relevant experimental techniques.



**Full project location:** Sutton Bonington Campus;

[Investigating the role of cytokinin in regulating drought tolerance.](#)

**Project Supervisor:** Anthony Bishopp

**School:** Biosciences

**Description:** Through the work of a recently graduated PhD student, we have identified that, in *Arabidopsis*, a specific cytokinin signalling component, AHP4, regulates root growth in water limited conditions. We also identified an orthologue of this protein as affecting root growth in water limiting conditions in maize. We, therefore, see this protein as a key regulator of drought response.

During this lab rotation, the student will investigate the effect that a wider number of cytokinin signalling components have on controlling root growth under stress.

- In weeks 1-2, the student will grow a range of mutants in cytokinin signalling components under low water availability.
- In weeks 3-4, the student will quantify root growth including measuring meristem size using confocal microscopy. We do not presently have any cytokinin mutants or reporters in maize.
- In weeks 5-9, the student will work with the PI and Co-I to design strategies to develop these, with the goal of amplifying the maize AHP4 promoter and cloning in into an entry clone.

**Location:** Sutton Bonington Campus

**Full project description:** Background: Water limitation provides a major constrain on agriculture. Some crops are better adapted to water stress environments, and such adaptations include changes in root architecture and anatomy, with deeper roots proving efficient at accessing deeper ground water. We have identified that the cytokinin response gene AHP4 controls the elongation of the primary root of Arabidopsis under water stress. We show that AHP4 is expressed in the phloem and modulates the flow of solutes into the root. We do not yet know the mechanism through which AHP4 regulates this process. The first objective is to understand what downstream pathways are regulated by AHP4. Through GWAS, together with our collaborators we have identified that the maize AHP4 orthologue is associated with increased lateral root growth under drought. The second objective is to determine whether AHP4 function is conserved between Arabidopsis and maize.

Work plan:

Year 1-2: Determine the mechanism through which AHP4 functions. We know that AHP4 is induced under drought. The student will test whether it is also induced under any other environmental stresses. We hypothesise that AHP4 functions by modulating cytokinin response. The student will test this by combining *ahp4* mutants with other known cytokinin mutants to look for changes in drought sensitivity. We do not know the downstream genes that AHP4 controls. The student will investigate these by performing RNASeq analysis of wild-type versus *ahp4* under drought stress. We would also like the student to develop an inducible over-expressor of AHP4.

Year 2-4: Is AHP4 function conserved with maize? Growing and transforming maize is more complicated and involved than Arabidopsis. Therefore, we need to carefully consider which analyses are done in maize. In the first instance, the student will clone the maize AHP4 orthologue and drive it in Arabidopsis under the AHP4 promoter to see if this can rescue the Arabidopsis *ahp4* mutant. This can be done fairly. The student will then prepare constructs for transformation into maize, these could include a transcriptional reporter and a CRISPR knockout.

Other possibilities: We already have the orthologous mutants to AHP4 in rice. The student will have access to this material and would be able to look at the role of these genes in a wider number of crop species. We are interested in evolution of cytokinin signalling generally, so would support the student in performing phylogenomic analyses of cytokinin signalling components.

Output and impact: We hope that this research identifies a new loci that can be exploited for developing or identifying crop varieties that can better withstand the low water conditions that are becoming increasingly common.

**Full project location:** Sutton Bonington Campus;

## Space Farming: Optimising resource use efficiency in Bioregenerative life support systems (BLSS)

**Project Supervisor:** Alexandra Burgess

**School:** Biosciences

**Description:** Using available plant and growth facilities at Sutton Bonington campus, this lab rotation will help form the foundation of the skills required in plant phenotyping and controlled environment agriculture, covering techniques spanning plant science and computer vision. The rotation will include experience in working with both duckweed- a small aquatic plant- and cereal or broadleaf crops. Candidates will have access to training for in silico canopy analysis including robot-aided image capture, 3D reconstruction and an introduction to deep learning, with access to state-of-the-art machines.

Other techniques include plant propagation and husbandry in controlled environments, growth assessments and, principles of photosynthesis analysis will be given. Comparisons between Earth based and Space based (i.e. BLSS) controlled environments will be explored via a combination of literature reviews and online training. In addition, candidates will be trained in plant physiological techniques including gas exchange and chlorophyll fluorescence. Remote sensing using spectroradiometry will also be accessible if desired.

Other, non-project specific skills will be taught during this time including critically reviewing papers during journal clubs, an introduction to scientific publishing and the editing process and resilient leadership training; all of which will be facilitated by the main supervisor.

**Location:** Sutton Bonington Campus

**Full project description:** Future long-term space missions will rely on the production of food on space crafts within bioregenerative life support systems (BLSS). These are highly controlled environments where all inputs, or growth 'resources', can be modified. Controlling environmental variables can provide numerous benefits due to the maintenance of optimal growth conditions, ultimately leading to increased productivity. Whilst resource-use efficiency (RUE) represents the baseline for the productive use of resources (including water, nitrogen, light, and land/space), it has yet to be fully optimised within controlled environments, either on Earth or in BLSS.

Due to restrictions on payload combined with a severely limited external supply of resources, BLSS should be conceptualised as 'closed' or 'circular' systems. Therefore maximising productivity and minimising waste is of utmost importance. Many features of high technology- focused controlled plant growth are similar on Earth and in Space. This includes a light source (usually light emitting diodes- LEDs), temperature and humidity regulation, and often soilless cultivation systems such as hydroponics.

One way to improve RUE in controlled environments is to use principles of RUE from field environments. For example, intercropping is an approach in which two or more crops are cultivated in close proximity at the same time. This enables RUE to be maximised as a result of each component plant having temporally distinct peak resource requirements, or through one plant providing a benefit or service to another (e.g. nitrogen fixation). Within the field, plants are also subject to wind-induced motion. Whilst little is known about the physiological impact, it is postulated that this movement may facilitate the distribution of light and mixing of gases throughout the canopy.

Within this project, routes to optimise RUE within BLSS will be identified. This will combine experimental work in controlled environment agriculture at Nottingham as well as a placement at the University of Adelaide, Australia as part of the joint research theme; 'Plants4Space'.

Specific objectives of the project are to:

1. Identify combinations of crops that can be cultivated within the same systems to maximise RUE.
2. Determine the potential role of flow-induced movement within controlled growth environments
3. Use process based modelling to optimise BLSS system design.

A multi-disciplinary approach will be used to meet these objectives, including both physical experimentation and in silico modelling. Plant physiological techniques will include chlorophyll fluorescence and gas exchange to capture and monitor photosynthetic productivity, whilst growth analysis will be performed using a combination of physical measurements and image analysis. During a placement in Adelaide, there will be the opportunity to use bespoke space-simulating equipment such as 1) a random positioning machine (to simulate microgravity), 2) radionuclide-based cosmic-ray emission facilities, and 3) potassium and phosphorus fertilisers leached from moon simulants. The combined data from both Nottingham and Adelaide will be used to design a BLSS by virtue of process modelling, which will inform system engineering design. This will be incorporated into sustainability assessments, starting with cost and environmental (life cycle assessment) and ending with circularity economy (CE) and Environmental, Social and Governance (ESG) assessments.

**Full project location:** Sutton Bonington Campus; University of Adelaide (placement);



## Phenotypic and molecular characterisation of rice *axr4* mutants

**Project Supervisor:** Ranjan Swarup

**School:** Biosciences

**Description:** As part of the rotation students' will be involved in analysis of rice *axr4* Crisper mutants and will be exposed to a number of routine and some specialised techniques in Molecular Cell Biology, plant phenotyping and plant physiology such as:

- Reporter gene studies
- In situ immunolocalisation
- Confocal Microscopy
- Lightsheet Microscopy
- In vitro plant culture
- Microtome sectioning
- PCR and RT-PCR



- Image processing packages including ImageJ and WinRHIZO

**Location:** Sutton Bonington Campus;

**Full project description:** Plant hormone auxin plays crucial roles in almost all aspect of plant growth and development. Auxin transport is carrier mediated and facilitated by auxin influx and efflux transporters. In Arabidopsis, auxin influx carriers are encoded by a small gene family within the Auxin-amino acid permease superfamily comprising four members AUX1, LAX1, LAX2 and LAX3. AUX1/LAX proteins are multi membrane spanning plasma membrane proteins. Sorting controls are crucial for the regulation of targeting of the plasma membrane (PM) proteins and thus they define various key processes including directional transport of auxin. Despite the importance of auxin transport in plant development, in contrast to the PIN auxin efflux carriers, sorting and PM targeting of their counterparts auxin influx carriers AUX1/LAX proteins is not well understood. In Arabidopsis, ER (endoplasmic reticulum) protein AXR4 has been shown to regulate the trafficking of AUX1 and LAX2. In the absence of AXR4, AUX1 and LAX2 are predominantly localised in the ER (Dharmasiri and Swarup et al, Science; Tidy et al 2023, Plant Physiology, In Press). AXR4 is a plant-specific protein and contains a weakly conserved  $\alpha/\beta$  hydrolase fold domain that is found in several classes of lipid hydrolases and transferases. We now have provided evidence that AXR4 is unlikely to act as a post-translational modifying enzyme as mutations in several highly conserved amino acids in the  $\alpha/\beta$  hydrolase fold domain can be tolerated and active site residues are missing. Our results suggest that AXR4 acts as an ER accessory protein which are special class of ER proteins that regulates targeting of their cognate partner proteins (Tidy et al 2023, Plant Physiology, In Press).

A rice homolog of AtAXR4 has been identified but it only shares a 30% similarity with AtAXR4 at the protein level and is predicted to localise to the chloroplasts. Using a functional complementation approach, we now show that OsAXR4 is localised to the ER in Arabidopsis and is able to restore Arabidopsis axr4 mutant phenotypes as well as can correct the targeting defect of LAX2 to the PM. A CRISPR-Cas9 mutant of OsAXR4 has been obtained. OsAXR4 mutant is agravitropic. We have previously shown that root hair elongation under low P requires AUX1 (Bhosale et al 2018, Nature Communication; Giri et al, 2018, Nature Communication) and the axr4 crispr mutant is also defective in root hair elongation response under low P. This shows that rice AXR4 behaves similar to AtAXR4 in regulating trafficking of auxin influx carrier. Interestingly, preliminary results show that rice axr4 mutants show some interesting above ground phenotypes including delayed germination, shorter stature, fewer tiller numbers, late flowering and defects in grain filling.

This project will investigate the molecular basis of defects in AXR4 Crispr mutant.

Aims and objectives.

1. Detailed characterisation of axr4 mutant
  - a. Root defects; b. Germination defects c. Shoot defects; d. Seed filling defects
2. Expression studies
 

Reporter lines will be created (AXR4Pro::AXR4-GFP and AXR4Pro::GUS) to investigate expression and localisation of AXR4 at organ, tissue, cellular and sub-cellular level.
3. Identification of AXR4 targets

A cross linking and proteomics-based approach will be used to identify potential novel targets of AXR4.

**Full project location:** Sutton Bonington Campus;

Environmental bioremediation using predatory bacteria – taking the first steps towards applications of *Bdellovibrio* against pathogens in farm waste.

**Project Supervisor:** Laura Hobley

**School:** Biosciences

**Description:** *Bdellovibrio* are predatory bacteria that prey on many Gram-negative pathogens, including WHO priorities *E. coli*, *Pseudomonas*, *Klebsiella* and *Acinetobacter*. Due to their predatory abilities, *Bdellovibrio* are proposed to have a variety of potential antimicrobial applications: medical treatments in humans and animals; in agricultural and food production to remove bacterial contamination, and in bioremediation of environments including sewage and farm waste (such as dairy slurry).

Our interest is in this final application. Slurry collected from dairy farms contains high concentrations of Gram-negative bacteria (including *Pseudomonas*, *Acinetobacter* and *E. coli*) many carrying AMR genes, which pose potential risks when spread onto fields of crops. Metagenomics analysis of slurry from the Sutton Bonington dairy farm indicates that *Bdellovibrio* spp are naturally present within slurry. The main questions to be answered in this project are:

- 1) Can we isolate a range of potential (pathogenic/AMR) prey bacteria from slurry, identify it phylogenetically, and characterise its antibiotic resistance profile?
- 2) Can we isolate predatory bacteria from the slurry, and confirm the mode of predation?
- 3) How many of our slurry prey bacteria can be preyed upon by the well-characterised type strain of *Bdellovibrio bacteriovorus*? How does this compare with our novel predatory isolates?

**Location:** Sutton Bonington Campus;

**Full project description:** Dairy farms in the UK produce approx. 67 million tonnes of manure per year, the liquid component of which is stored as slurry before being spread onto crop fields. Within the slurry there are high numbers of Gram-negative bacteria, including many pathogens (*Pseudomonas*, *Acinetobacter*, *E. coli*) identified as WHO priority species. Many of the bacteria in slurry carry antibiotic-resistance genes (ARGs). These pose two different threats: the presence of the pathogens themselves which pose a risk to both human and animal health, and the presence of the ARGs, which are often found on mobile genetic elements allowing them to easily be transferred between bacterial species (both pathogenic and non-pathogenic).

*Bdellovibrio* are predatory bacteria that prey upon a wide range of Gram-negative bacteria, including many pathogenic species (*E. coli*, *Acinetobacter*, *Klebsiella* and *Pseudomonas*). Predation occurs by the *Bdellovibrio* entering the prey cell, and using the prey cell contents as nutrients for growth and replication, degrading all the prey cell DNA, before multiple progeny *Bdellovibrio* are released to continue the predatory cycle. As such, *Bdellovibrio* have been proposed to have a wide range of potential applications – here we are focussed on their potential bioremediation uses in dairy slurry, where they have the potential to not only reduce the population sizes of the Gram-negative bacteria, but to also reduce the number of ARGs present in slurry.

We have preliminary data showing that *Bdellovibrio* are present within slurry, and have recently shown that 80 different Gram-negative bacteria we isolated from slurry can all be preyed upon by the lab strain of *Bdellovibrio*. This project will build upon this, and the experiments proposed for the rotation project. The main questions that we aim to answer through the PhD project are:

1) What predatory bacteria are in dairy slurry and how phylogenetically diverse are they? Predatory bacteria exist beyond the *Bdellovibrio* spp, so isolating and characterising any predators within the slurry will allow us to get a greater understanding of types of predation taking place within the slurry populations. We will adapt existing isolation techniques to enable us to isolate a range of predatory bacteria.

2) Of the bacterial populations within slurry how many can be preyed upon by the predators also in slurry. How efficient is this predation? Do the predators exhibit prey-preference when put in cultures with multiple prey species? This will be in lab-culture conditions, but will lead into mini-slurry tank experiments in aim 3.

3) What will happen if we add large numbers of predatory bacteria into actual slurry? We will use small-scale mini-slurry tanks to replicate what happens in the large tanks found on farms. We will take fresh slurry samples from the dairy farm, and spike them with predatory bacteria, both with and without particular prey that we are interested in, then use culture-dependent and metagenomics methods to analyse the effects on the bacterial populations (and the number of ARGs) within these mini tanks.

Together these aims will elucidate the potential of predatory bacteria in the bioremediation of dairy farm waste.

D. Negus, C. Moore, M. Baker, D. Raghunathan, J. Tyson, R.E. Sockett. (2017) "Predator Versus Pathogen: How Does Predatory *Bdellovibrio bacteriovorus* Interface with the Challenges of Killing Gram-Negative Pathogens in a Host Setting?" *Annual reviews of Microbiology* 71:441-457

Baker M, Williams AD, Hooton SPT, Helliwell R, King E, Dodsworth T, Baena-Nogueras RM, Warry A, Ortori CA, Todman H, Gray-Hammerton CJ, Pritchard ACW, Iles E, Cook R, Emes RD, Jones MA, Kypraios T, West H, Barrett DA, Ramsden SJ, Gomes RL, Hudson C, Millard AD, Raman S, Morris C, Dodd CER, Kreft J-U, Hobman JL and Stekel DJ. 2022. Antimicrobial resistance in dairy slurry tanks: a critical point for measurement and control. *Environment International* 169: 107516

**Full project location:** Sutton Bonington Campus;

[Microbiomes of beneficial arthropods in agricultural systems and their influence on biocontrol](#)

**Project Supervisor:** Sara Goodacre

**School:** Life Sciences

**Description:** The diversity of micro-organism communities that are hosted by invertebrate species remain poorly understood. This rotation will establish methods for characterising microbial diversity and determining the likely relationship between these micro-organisms and particular spider hosts, which perform an important role as predators of insect pest species in agricultural systems.

The rotation will be based largely in the Goodacre lab, with regular trips to visit the other two supervisors. The aims are to learn how to:

1) Molecularly characterise microbial species using PCR and next generation sequencing technology, using a metabarcoding approach

2) Analyse molecular sequences to place these data in a phylogenetic context, and to test for the presence of genes of known physiological/ecological function

3) Measure biocontrol potential (e.g. ability to hunt and find prey) of field-sampled invertebrates taken from farmed environments, in the presence or absence of insecticides

The overall aims are to introduce the different techniques that will be used in the wider PhD, and to establish good communication with the colleagues and expertise available in the different supervisor laboratories.

**Location:** University Park;

**Full project description:** The diversity of microbial communities hosted by many invertebrate populations remain poorly understood and uncharacterised, despite evidence that these 'unseen' microbial passengers can influence traits such as the potential for invertebrates to act as vectors of disease (e.g. dengue transmission by mosquitoes is altered by intracellular microbes of the Rickettsia family). Beneficial invertebrates such as spiders, which act as biocontrol agents of a wide range of pest species in agricultural fields, carry a diverse range of microorganisms, some of which are transmitted vertically and others horizontally. Some of these have already been implicated directly in the spider host's ability to survive exposure to insecticides used in agricultural areas. Others are known to influence genetic connectivity between populations, thereby influencing the possibility of sharing beneficial traits, such as host resistance to disease. The relationships between most of the microbial species present and their host in terms of mutualistic, parasitic or symbiotic interactions, and the mechanisms through which they exert their influence, however, remain incompletely understood.

In this project we will explore the natural communities of microorganisms found in populations of spiders commonly in agricultural settings. We will further investigate host-microbe interactions in order to determine the role of microorganisms in shaping diversity within and among host populations and the potential for biocontrol of agricultural pest insects. In order to achieve our aim we will study both a) naturally occurring populations of the spider host and b) populations where we have manipulated microbial populations using antibiotic treatment. We will molecularly characterise microbial species using Next Generation Sequencing technologies, using a metagenomic approach. We will target genes of known function that give us insights into the functional diversity of microorganisms as well as characterising genes known to be phylogenetically informative. We will compare our microbial data with the findings from laboratory studies of the phenotypic consequences of infection. We will include in our laboratory trials application of two types of insecticide that are commonly applied in agricultural settings (pyrethroids and neonicotinoids). These two families of agent are both known to be highly detrimental to spiders, which are not their intended targets. Preliminary data indicate that there is an interaction between particular types of microbial species and the extent of the response to insecticide exposure. We will establish in our experiments whether host-microbial interactions likely influence survival following insecticide exposure, with consequences for the potential for naturally occurring biocontrol potential of pest by resident spider populations.

We will combine in our project expertise provided by three partners 1) the SpiderLab in the School of Life Sciences at the University of Nottingham, which has experience in the study of the molecular genetics of these types of invertebrate and their micro-organisms (supervisor Goodacre) 2) expertise in the study of invertebrate pest-control behaviour in the School of Animal Rural & Environmental Sciences (supervisor Martin) and 3) the computational biology and bioinformatics expertise, particularly in microbial genomics, metagenomics and host pathogen interactions, in the School of Veterinary Medicine and Science (supervisor Blanchard).

**Full project location:** University Park;Brackenhurst Campus;Sutton Bonington Campus;

Harvesting novel alleles from the *Triticum timopheevii* genome for bread wheat improvement

**Project Supervisor:** Surbhi Grewal

**School:** Biosciences

**Description:** The 9 week mini-project will primarily focus on training of key techniques and gaining experience in experimental design and laboratory techniques. This will involve working with three aspects of the wheat pre-breeding programme namely wide crossing techniques, molecular marker development and cytogenetics, all of which are key to the PhD project.

- Weeks 1-3: a) Cross bread wheat with the wild relative species to obtain F1 plants. This will give the student key glasshouse and plant management skills in addition to an insight into wheat spike morphology and the techniques of emasculation and pollination. b) Germinate plants for a wheat rust screening experiment.
- Weeks 4-6:a) Use bioinformatic methods to exploit the genome assembly for SNP discovery and development of KASP markers in the target genomic region. Validate the KASP markers by testing them on parental species using a high-throughput genotyping platform incorporating the PCR technique. b) Inoculate plants with rust spores for disease resistance screening.
- Weeks 7-9: a) Extract DNA from the wild relatives and related wheat progenitor species to develop fluorescent probes. Use the probes to characterise the wild relative species via GISH technique. b) Score plants for infection severity to identify potentially resistant lines.

**Location:** Sutton Bonington Campus;

**Full project description:** Background: Due to modern breeding practices, relatively little genetic variation is available in modern wheat varieties for breeders to develop adapted cultivars with increased yield potential and tolerance to abiotic and biotic stresses. However, the close wild relatives of wheat represent a practical gene pool for use by breeders. These wild relatives are being exploited as sources of novel genetic material by the pre-breeding programme being undertaken at the Nottingham BBSRC Wheat Research Centre (WRC), where small segments of various wheat wild relative species are being introgressed into bread wheat to create stable introgression lines that can be used for downstream trait analysis.

*Triticum timopheevii* is one such species shown to carry novel genes conferring resistance to diseases, such as wheat rusts and Fusarium Head Blight<sup>1</sup> and desirable flowering morphology traits for hybrid wheat production such as smaller pollen grains in addition to various other quality traits such as improved grain mineral content. At WRC, we have generated and characterised over 150 wheat-T. *timopheevii* introgression lines that can be exploited for such traits<sup>2</sup>. To fully unlock the potential of T. *timopheevii* for wheat improvement and obtain useful alleles for various traits, an annotated high-quality reference genome for this species has very recently been generated by the WRC (to be published). Comparative genomics, using this reference genome, with the publicly available wheat reference genome will uncover the effect of domestication and deliver novel alleles for numerous desired traits.

**Aims and Objectives:** In this project, we aim to exploit the germplasm at WRC (existing and new) containing small segments of *T. timopheevii* for up to three desired traits (depending on time and resources), namely, leaf rust resistance, small pollen grains and improved grain zinc (Zn) and grain iron (Fe) content for the identification of QTL(s) and candidate genes using various approaches. This will be carried out through extensive phenotyping via glasshouse screening and field trials as well as comparative genomic techniques.

Year 1 and 2:

1. Screen a panel of *T. timopheevii* introgression lines that contain the entire genome of the wild species for the desired traits to identify the target introgression(s) for each trait.
2. Create an F2 segregating population for each trait by crossing the introgression line(s) carrying the desired trait with wheat to create the F1 plant and further self-fertilisation to segregate the segment in an F2 population.
3. Create new wheat-*T. timopheevii* introgression lines by carrying out a backcrossing programme with new *T. timopheevii* accessions characterise the new germplasm using bioinformatic, molecular and cytogenetic tools such as whole genome sequencing, KASP markers and genomic in situ hybridisation (GISH), respectively (to be carried across years 3 and 4 as well).

Years 3 and 4:

1. Screen the F2 population for the desired trait to establish a link between the desirable trait and the segment from *T. timopheevii*.
2. Simultaneously, lines of interest will be sent for field trials to WRC collaborators at the USDA in Kansas, CIMMYT in Mexico and commercial wheat breeders in UK.
3. Comparative bioinformatic analyses in the target genomic regions using the *T. timopheevii* and wheat genome assemblies will be carried out to identify QTLs and candidate genes for each desired trait.

Supporting Framework

Previous BBSRC funded programmes such as Designing Future Wheat (DFW) and International Wheat Yield Programme (IWYP) have provided the pilot data on the desirable traits present in the WRC wheat-*T. timopheevii* introgression lines.

References

1. Steed, A, et al. 2022. *Front. in Plant Sc.* 13:943211.
2. King, J. et al. 2022. *Front. in Plant Sc.* 13:919519.

**Full project location:** Sutton Bonington Campus;

[Defend or Welcome: Evolution and regulation of plant cell walls to control microbial colonisation.](#)

**Project Supervisor:** Ute Voss

**School:** Biosciences

**Description:** This DTP rotation and project will address how biophysical plant cell wall properties evolved to control microbial colonisation. Our interdisciplinary programme comprises novel and unique equipment, recently developed microscopy tools, artificial intelligence-based data and image analysis and is supported by the broad range of expertise within the supervisory team

The rotation will introduce the student to a broad range of techniques that will be useful for their future PhD and Research career:

- Week 1: Health and Safety and Lab training
- Week 2: Phenotyping and microscopy training
- Week 3: Imaging and Image analysis training
- Week 4-6: Genotyping and microscopy
- Week 6-8: Phenotyping, Microscopy & image analysis
- Week 9: Write-up and data interpretation

**Location:** Sutton Bonington Campus

**Full project description:** Plants colonised land about 500 million years ago and paved the way for the evolution of today's complex terrestrial ecosystems. Diversity of terrestrial plant ecosystems rests on a complex web of interactions between plants and soil microbiome. The importance of soil microbiome for development of soils, plants, and ultimately Earth's climate has long been acknowledged, but complexity limited our understanding of detailed mechanisms how soil microbiome and plants interact to achieve a successful symbiosis that leads to establishment of diverse and rife ecosystems.

The plant hormone auxin is a key regulator of plant development. The mechanism of auxin action is highly complex, and despite considerable research effort little is known about fundamental mechanisms of its action. Developmental "decisions" are regulated by an interplay between auxin metabolism, signalling, and transport, with intercellular auxin gradients playing the key role. Auxin also regulates cell wall stiffness which has profound impacts on the ability of beneficial and pathogenic microbes to colonise plants. The ability of microbes to synthesise or degrade auxin is essential for the microbiome-plant symbiosis. This project sets out to uncover auxin-dependent plant-microbiome interactions, leading to new fundamental knowledge. The key enablers of this research are the new sequencing, high-throughput techniques as well as artificial intelligence-based approaches pioneered by the supervisors.

How plants regulate their cell wall stiffness in response to rhizome microbiota is largely unknown. We will address this fundamental question using the plant model *Physcomitrium patens* and *Arabidopsis thaliana*. The moss *Physcomitrium* belongs to the bryophytes, the most basal land plants. Auxin- and cell wall related mechanisms appear genetically simpler in moss compared to flowering plants such as *Arabidopsis*. We will investigate how auxin regulates cell wall stiffness to control microbe colonisation using a multidisciplinary and innovative approach combining molecular biology, microscopy, biochemistry, and mathematical modelling. We will analyse cell walls of root hair (*Arabidopsis*) and rhizoid cells (*Physcomitrium*) as they are fast growing cells. In Nottingham we have a unique combination of equipment and facilities allowing us to address this innovative and timely question by answering:

Objective 1. How does local auxin impact cell wall stiffness in a dose dependent manner across tissues? Comparing wildtype with auxin metabolism- and cell wall mutants for cell wall stiffness with the world-unique Biofluidic Microscope.

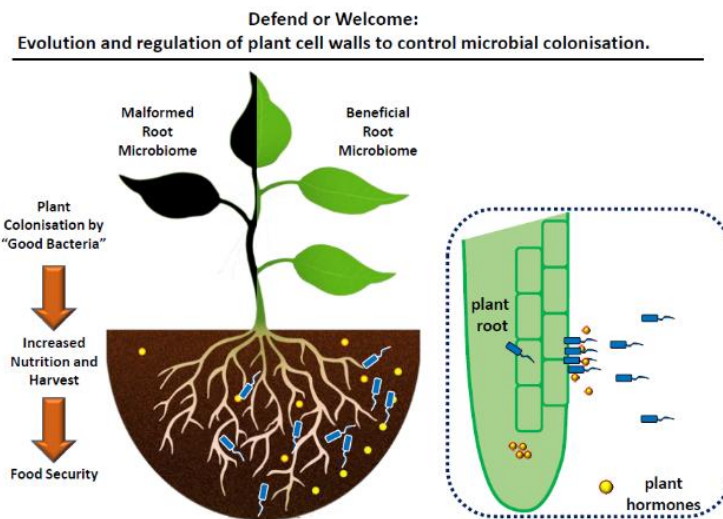
Objective 2. How does auxin regulated cell wall stiffness impact microbial colonisation? We will visualise and quantify plant microbe colonisation in above-described mutants using auxin producing and -degrading root colonising microbes using light sheet microscopy.

Objective 3. How do plants control cell wall properties to control microbial colonisation? Generating and analysing an RNAseq dataset will identify candidate genes targeted by

colonising microbes to modify cell wall composition and stiffness. Implementing published datasets will generate a gene regulatory network model to be validated during the PhD.

**Full project location:**

Sutton Bonington  
Campus;



Environmental pollen resilience:- understanding the impact of heat and light stress on pollen development to maintain yield under environmental stress.

**Project Supervisor:** Zoe Wilson

**School:** Biosciences

**Description:** Pollen formation is critical for plant reproduction and food production and is thus essential for plant breeding and food security. Nevertheless, pollen development is highly susceptible to environmental stress, which can result in a lack of viable pollen and sterility; variation in pollen production and fertility is frequently seen between species and in different environments. This poses a significant problem for maintaining future crop yields with increasingly volatile environmental conditions. Control of pollen wall formation, and thus viable pollen development, is regulated through a series of transcription factors, which act in the tapetum cell layer in the anther. These transcription factors have been shown to respond differently to altered temperature and light, and to be modified by SUMOylation. SUMOylation has been shown to coordinate growth control under changing environmental conditions by directly modifying the activity of major transcriptional regulators in plants. This rotation will involve the analysis of mutants that are modified in their ability to SUMOylate key transcription factors linked with pollen development. These will be analysed under different environmental stress conditions to establish the link between environmental sensing and pollen development.

**Location:** Sutton Bonington Campus;

**Full project description:** Pollen formation is critical for plant reproduction and breeding, and thus for the production of crops and food. Ensuring successful reproduction is therefore essential for food security. However, pollen development is highly sensitive to environmental stress, which can result in a lack of viable pollen and sterility under different stressful environments. This poses a significant problem for maintaining future crop yields due to increasingly volatile environmental conditions and climate change. Pollen formation is controlled by a maternal cell layer within the anther



called the tapetum, which encloses the developing pollen. This cell layer functions both as a coordinator and as a factory, controlling pollen development, but also the manufacture and secretion/release of pollen wall materials.

A number of transcription factors (TF), which are expressed within the tapetum regulate this process, in particular 5 bHLH TFs are involved. These bHLH TF proteins interact with each other and also regulate the expression of each other, as well as distinct sets of downstream genes. This means there are a series of complex, potentially competitive, interactions resulting in feed-forward and feed-back regulatory loops, which ensure that the correct levels of gene expression occurs at the right time and place. This is critical to ensure the formation of the intricate and resilient pollen wall, and viable pollen. We have shown that three of these bHLH TF are labelled by Post- Translational Modifications via Small Ubiquitin-like Modifier (SUMO) tags. SUMOylation is a reversible process known to modify protein activity, localisation or stability, particularly in response to stress. It is currently not known how these modifications impact tapetum function and pollen viability, however we have also found that these TF respond to growth under different environmental conditions (heat and light). This has identified a mechanism whereby plants can respond to changing environments and abiotic stress during flowering to ensure that pollen formation and fertility is maintained, under natural, variable environmental conditions. Such adaptation will be extremely valuable to maintain crop yield in volatile environments and climate change.

This project will investigate the control and impact of these TF protein-protein competitive interactions and establish the role of SUMOylation on their activity and function. This will be conducted through a combination of molecular genetic and biochemical analyses of the network, and the impact of SUMO tagging of these proteins on TF activity and interactions, and the downstream regulatory network will be investigated. This will enable characterisation of how plants ensure that pollen formation and fertility is maintained under changing environments and abiotic stress. This project will focus on the model plant *Arabidopsis* to capitalise upon available molecular genetic tools to rapidly test this network. However, we have also been working on the equivalent genes in barley which show a high level of conservation; this project will build on these data to help establish the role of SUMOylation in the barley tapetum gene network to enable the rapid deployment of this knowledge to target environmental resilience in pollen development in crops.

**Full project location:** Sutton Bonington Campus;

[Metaphenomics as a tool for assessing soil health](#)

**Project Supervisor:** Marcello Di Bonito

**School:** School of Animal, Rural and Environmental Sciences (NTU)

**Description:** The activities will be carried out across the two institutions and provide an understanding of the challenges with the soil metaphenomic approach.

- NTU – ARES - Soils with different histories and landuse (e.g., pasture, no tillage, tillage, and forest soil as control) will be assessed and defined as a habitat in the field. Soils will be sampled, prepared, archived and quality controlled to measure key physical and chemical indicators, including: structure, texture, temperature and water content; C, N, and S pools and fraction (by Organic Elementar analyser vario EL cube).
- UoN – SB - Classical soil microbiological techniques will be utilised to determine soil biological processes (e.g. soil enzyme assays), microbial biomass (using

chloroform-extraction methods followed by chemical analyses for microbial C, N and P), ergosterol measurements to evaluate fungal presence and arbuscular mycorrhizal fungal quantification through root staining procedures. Metabolic potential will be measured using Ecoplates as an introduction to soil metabolic processes.

- NTU – SST - Sequencing libraries will be prepared from DNA extracted from soils samples by PCR of the V3V4 region of 16S rDNA, and quality control checked using an Agilent tape station. Microbial taxa will be classified using specialist R metataxonomic packages.

**Location:** Sutton Bonington Campus;Brackenhurst Campus;Clifton Campus

**Full project description:** This project will investigate the use of metaphenomics approach to provide a better understanding of complex soil systems in different environmental conditions. Metaphenome is defined as the product of expressed functions encoded in microbial genomes (metagenome) and the environment (resources available; spatial, biotic and abiotic constraints). The soil metaphenome is dependent on the combined genetic potential encoded by the soil member genomes, the physiological status of the member populations, their access to resources, contact with other organisms and signalling molecules, combined with their genetic capacity to respond to environmental cues. We will use an integrated multiomic approach to evaluate the physiological responses produced by the soil's microbiome to external conditions as well as their pathway of interactions with plants and/or microorganisms. We will examine a variety of soil types, according to their classification, landuse, and health status by using state of the art untargeted Mass Spectrometry, Nuclear Magnetic Resonance (NMR) spectroscopy and Stable Isotope techniques and mapping these characteristics across the soil spectrum, both spatially, temporally and functionally.

Soil metaphenomics has recently demonstrated the potential to reveal the complex molecular network and metabolic pathways operating in the soil microbial communities and a means of evaluating soil function. It is an important area of research in ecology and environmental sciences that can be applied to agricultural sciences because it could offer tools and answers for soil conservation, food security (e.g., crop yields and nutrient value), climate change (e.g., soil respiration), greater biogeochemical cycles (C, N, micronutrients), and ultimately ecosystems functions (soil health). However, soil metaphenomics presents unique challenges, because soil spatial complexity and highly dynamic distribution of nutrients (C, N, S, and micronutrients) and other resources can result in hot spots for growth of microbial consortia, for example within micro-aggregates and/or the rhizosphere. Understanding the fine scale distribution of microbes and resources is required to predict species physiology and metabolic interactions among community members, that comprise the collective soil metaphenome.

This project addresses several existing knowledge gaps and research priorities all the above themes.

In order to investigate the use of metaphenomics on soils, we need to deliver three aims:

1. Understand how the use of metabolic models can help to predict phenotypic responses of micro-organisms to different environmental conditions.
2. Build relevant databases to fill gaps on metabolite composition of soils.
3. Begin to map metabolite composition of soils across different soil types and conditions.

The proposed project/proof of concept will use sensitive mass spectrometry platforms to predict the identities of metabolites, which will be combined with advanced

computational approaches, and help increasing the number of soil metabolites assignments in databases.

We will achieve our aims by carrying out three objectives:

1. Test the validity of flux-based analysis (FBA) on different types of soils to decipher specific metabolic pathways (in specific microbial communities).
2. Assess stable isotope probing (SIP) and multi-omics approaches to assess functions carried out by members of soil microbiome that result in the soil metapenome.
3. Assess soil-specific genera/species presence by sequencing regions of 16S rRNA.

**Full project location:** Brackenhurst Campus; Clifton Campus; Sutton Bonington Campus;

### Methane microbiology

**Project Supervisor:** Dr Ellen Nisbet

**School:** Biosciences

**Description:** Methane emissions are at a global high, and are having a major impact on climate change. The UK has committed to reducing methane emissions by 50% by 2030. Although much work is being done to cut emissions from the oil and gas industries, agricultural methane emissions are more intractable. In the UK, the major emitters of agricultural methane are cattle, manure heaps, and biodigesters.

This project aims to understand methane emissions from agricultural sources. Methane is produced by methanogens (archaeobacteria), and removed by methanotrophs (archaeobacteria and bacteria). Therefore, understanding the microbiome of farmed cattle is extremely important in mitigating methane emissions.

The project will involve manure sampling, methane emission measurement from cattle, DNA extraction and sequencing and bioinformatic analyses. Full training will be given. In addition, there will be the possibility to analyze previously obtained data from both British and Kenyan cattle. The microbial content will be compared with the methane emission rates. The co-supervisor on this project is based at Royal Holloway, and the student will be able to visit. This will allow them to learn how methane measurements are carried out.

**Location of Lab Rotation:** Sutton Bonington Campus;

**Full project description:** The growth in atmospheric methane in 2021 was the greatest on record. This growth was not expected in the 2015 Paris Agreement, where countries agreed to limit global temperature increases to 1.5C. Cutting methane emissions is a key UN and UK goal. The UK has signed the Global Methane Pledge, aiming for a 30% reduction in methane levels by 2030. Although much work is being done to cut emissions from oil and gas industries, agricultural methane emissions are more intractable. In the UK, the major emitters of agricultural methane are cattle, manure heaps and biodigesters.

This cross-disciplinary project will examine the methane microbiology and emission fluxes from farming. This involves both DNA studies and atmospheric chemistry, to target emission mitigation strategies. This project brings together microbiologists at UoN, with expertise in environmental microbiology (Nisbet) and metagenomics of farm slurry tanks (Hobman) with the world-leading expertise of the atmospheric chemistry laboratory at Royal Holloway (Fisher), who have been at the forefront of global methane monitoring for the past 30 years.

## Project

Cattle breath and manure wastes produce roughly 115 million tonnes of methane annually, out of total emissions around 550-600 million tonnes (Saunois et al, 2020). Anaerobic decay in manure tanks and ponds means manure stores are also major methane sources (Cárdenas et al. 2021). These are prime targets for emission mitigation.

To quantify methane emissions from UK manure stores, and to design targeted mitigation strategies, the PhD student will:

- 1) quantify methane emissions from manure stores; and
- 2) identify the underlying microbiology, using next generation sequencing.

The project will involve sampling from UK cattle, DNA extraction and bioinformatics, searching for methanogens (archaebacteria which produce methane) and methanotrophs (prokaryotes which consume methane). There will also be the opportunity to work with the team at RHUL, who sample methane worldwide, in order to learn about methane sampling and measurement in other environmental niches.

**Full project location:** Sutton Bonington Campus;