BSPP 2024 Undergraduate Vacation Bursary Fund Application, Contents

Funding Form - BSPP Undergraduate Vacation Bursary Fund Application : Entry # 1869

Title of Project

Tri-partite interactions between *Fusarium graminearum*, the bird cherry-oat aphid and Barley Yellow dwarf virus on wheat

This project is going to be...

Experimental (lab/field based)

Full Name of Supervisor

Rumiana Ray

Institution Department and Address

University of Nottingham
School of Biosciences, Sutton Bonington campus LE12 5RD
United Kingdom

Full name of the day to day supervisor and/or arrangements for supervision

The student will interact and receive training by a team of scientists in the Ray lab including Dr Amma Simon, Dr. Ying Lan, Dr Dasuni Jayaweera and Prof Rumiana Ray. Day to day supervision will be provided by Dr Amma Simon and Prof Rumiana Ray.

Date of Project Commencement

01/07/2024

Duration (weeks)

10

Brief Description of Project

Fusarium head blight (FHB) disease is caused by the fungal pathogen *Fusarium graminearum*, which infects the ears of cereal crops including wheat. Infection can result in reduced grain weight and quality, and the accumulation of mycotoxins which are harmful to humans and animals. FHB is a significant disease causing up to 50% wheat yield loss in addition to grain lost due to mycotoxin contamination costing up to €3billion to the agro-economy.
The bird cherry-oat aphid (*Rhopalosiphum padi*) is a major insect pest in Europe that feeds on cereal crop leaves and stems. This aphid reduces wheat yield by feeding on plant sap and transmitting viruses such as the Barley Yellow Dwarf Virus (BYDV). Symptoms of BYDV include reduced root and shoot growth, and leaf yellowing.
***F. graminearum* and *R. padi* share the wheat host, and individually can have detrimental effects on wheat food security, however, little is known about their host-mediated interactions. As the pathogen and the pest occupy different plant ecological niches, direct resource competition is unlikely whilst the systemic effects of *R. padi* infestation on *F. graminearum* and vice versa remain poorly understood. In this project, the systemic effects of non-viruliferous and viruliferous *R. padi* on the virulence of *F. graminearum* and FHB disease severity will be investigated. Simultaneously, the effects of *F. graminearum* on the fitness of *R. padi* will be determined together with viral disease progression.**

FHB visual disease assessments will be conducted to determine the effects of *R. padi* infestation on FHB disease progression. Aphid life history traits and BYDV assessments will also be carried out simultaneously to assess the effect of *F. graminearum* infection on BYDV disease symptom expression and *R. padi* development and reproduction. The student will perform DNA extractions to quantify the amount of *F. graminearum* DNA in wheat ears using qPCR. The effect of *F. graminearum* on BYDV will be determined by RT-PCR using BYDV specific primers.
This multi-disciplinary project will provide the student with virology, microbiology, and entomology related laboratory experience. Throughout the project, students will gain and refine insect handling, visual disease assessment and molecular biology skills. Students will use a variety of statistical software for data analysis.

Recommended reading for the project

1. DRAKULIC, J., BRUCE, T. J. A. and RAY, R. V., 2017. Direct and host-mediated interactions between Fusarium pathogens and herbivorous arthropods in cereals. Plant Pathology. 66(1), 3-13
2. JASSY DRAKULIC, OLUBUKOLA AJIGBOYE, RANJAN SWARUP, TOBY BRUCE and RUMIANA V. RAY, 2016. Aphid infestation increases *Fusarium langsethiae* and T-2 and HT-2 mycotoxins in wheat: Aphids increase T-2 and HT-2 toxins in wheat Applied and Environmental Microbiology. 82, 6548-6556
3. JASSY DRAKULIC, MOHD HAZIQ KAHAR, OLUBUKOLA AJIGBOYE, TOBY BRUCE and RUMIANA V. RAY, 2016. Contrasting Roles of Deoxynivalenol and Nivalenol in Host-Mediated Interactions between *Fusarium graminearum* and *Sitobion avenae* Toxins. 8(12), 353
4. JASSY DRAKULIC, JOHN CAULFIELD, CHRISTINE WOODCOCK, STEPHEN P. T. JONES, ROBERT LINFORTH, TOBY J. A. BRUCE and RUMIANA V. RAY, 2015. Sharing a host plant, wheat (*Triticum aestivum*), increases the fitness of *Fusarium graminearum* and Fusarium head blight (FHB) severity but reduces the fitness of grain aphids (*Sitobion avenae*): *Fusarium graminearum* infection renders wheat repellent to aphids Applied and Environmental Microbiology. 81(10), 3492-3501

Funding Form - BSPP Undergraduate Vacation Bursary Fund Application : Entry # 1865

Title of Project

Exploring hexaploid wheat GE lines defective in key aspects of fructan biosynthesis as a new way to reduce Fusarium head blight susceptibility

This project is going to be...

Experimental (lab/field based)

Full Name of Supervisor

Wanxin Chen

Institution Department and Address

West Common
Harpenden, Hertfordshire AL5 2JQ
United Kingdom

Full name of the day to day supervisor and/or arrangements for supervision

Wanxin Chen, will supervise and train the student on all computational and lab aspects of the project which includes:
• Training in Fusarium inoculation of plants, photography and quantitative disease assessment of inoculated wheat samples, statistics and data analysis.
• Training in sterile techniques, media preparation and fungal culture maintenance.
• Training in DNA sequence analysis with 'Geneious' software.
• Training in the theory behind GE in wheat.
• Training in sampling of wheat leaf material and genomic DNA.
• Training in molecular biology to genotype wheat plants, including PCR and gel electroporation.
Wanxin Chen has already genotyped and selected nine wheat Genome-Edited (GE) lines with stop codons introduced into the 1st exon of the 6-SFT gene, encoding an important Fructan biosynthesis enzyme. The student will continue to help to genotype the generation (T2) by PCR analyses. The confirmed homozygous T2 plants will be phenotyped by Fusarium inoculation into wheat spikes, followed by disease assessments. Wanxin Chen will have daily meetings to plan and check on progress and fortnightly meetings with the student and team leader Prof. Kim Hammond-Kosack to review the entire project's progress.
The student will interact with the PhD other student, Post-Docs and staff scientists in the lab and attend the weekly wheat pathogenomics team meetings.

Date of Project Commencement

01/07/2024

Duration (weeks)

8

Brief Description of Project

Fusarium head blight (FHB), caused by the fungus *Fusarium graminearum* (Fg), is one of the most destructive diseases of wheat due to yield losses, reductions to of grain quality and mycotoxin contamination in epidemic years. Lack of germplasm with good FHB resistance makes breeding wheat to be resilient to this global biotic threat particularly challenging. In cereal/ grass species, fructans are important carbohydrate storage compounds synthesised from sucrose with the help of three enzymes: 6-SFT, 1-SST and 1-FFT. Our previous studies using virus induced transient gene silencing indicated that fructans could act as host susceptibility factors contributing to FHB disease severity (RRes, KHK team unpublished). The 6-SFT and 1-SST knock-out mutant liness in the Cadenza background were generated using genome-editing (GE). Mutants with deletions in the three 6-SFT homoeologues on 4AL, 7AS and 7DS, which lead to a frame shift and a gained stop codon, were identified. Phenotyping of uninoculated T1 triple homozygous GE lines under controlled environment revealed most of them showed no remarkable difference in plant growth comparing to controls, but some lines produced significantly larger grains. Simultaneously, double homozygous Cadenza TILLING mutants of 4AL 7DS, 7AS 7DS and 4AL 7AS have been generated. Currently, these double mutant lines are being backcrossed with wildtype Cadenza to reduce the mutation background in the genome. The BSPP bursary student will have the opportunity to genotype the TILLING backcross lines and fine phenotype selected homozygous GE fructan biosynthesis pathway mutant lines for FHB disease formation under controlled environment conditions by using time-course and quantitative analyses as well as by measure fructan content pre and post Fg infection using HPLC analyses. Rothamsted Research has a multidisciplinary research environment. The appointed student will learn how to extract genomic DNA from wheat, undertake PCR analyses, prepare samples for Sanger sequencing, and undertake sequence alignment analyses using the Geneious software. For the phenotyping, the student will learn sample preparation for fructan measurement, how to aseptically culture the Fusarium fungus in vitro, prepare spore suspension cultures for

inoculation, undertake Fusarium floral spike and coleoptile spore inoculations using wheat plants at anthesis and young wheat seedlings, respectively, undertake quantitative followed by disease assessments over time-courses and calculate AUDPCsin seedling and adult plants. In addition, the student y will learn how to work under sterile conditions, use various microbiology techniques, photograph quantitatively assess FHB disease symptoms over time-courses, use a light and binocular microscopy, undertake experimental design, complete various data analysis and results interpretation analyses, give an oral presentation on this research project and write a report for the BSPP Newsletter.

Recommended reading for the project

1. Dweba C et al, (2017) Fusarium head blight of wheat: Pathogenesis and control strategies; Crop Protection, DOI: 10.1016/j.cropro.2016.10.002
2. Cimini S et al, (2015) Fructan biosynthesis and degradation as part of plant metabolism controlling sugar fluxes during durum wheat kernel maturation; Frontiers in Plant Science, DOI: 10.3389/fpls.2015.00089

Funding Form - BSPP Undergraduate Vacation Bursary Fund Application : Entry # 1864

Title of Project

Exploring the Mechanisms of Bacterial Pathogen Entry and Colonization in Plants through Wounds

This project is going to be...

Experimental (lab/field based)

Full Name of Supervisor

Pierre Buscaill

Institution Department and Address

Life Sciences Building
White, White British, Bristol BS8 1TQ
United Kingdom

Full name of the day to day supervisor and/or arrangements for supervision

day to day supervisor: Pierre Buscaill
supervision: In addition to regular meetings and specialized training provided by me, a lab manager will offer specific training and support in the use of common lab equipment. Our lab shares space with another research group focusing on plant immunity, providing daily opportunities for interaction with their team.

Date of Project Commencement

17/06/2024

Duration (weeks)

6

Brief Description of Project

How bacterial pathogens enter and colonise plant via wounds?
Wounding of plants by hard wind, hail, heavy rain, sand storms, and frost is common in nature. Many plant pathogens take advantage of this opportunity to infect plants. Thus, it is crucial to understand the molecular mechanisms involved in wound entry to prevent disease outbreaks. Despite its importance, these mechanisms remain poorly investigated.
Using the model bacterial pathogen *Pseudomonas syringae* pv. *syringae* DC3000 lacking the HopQ1-1 effector (PtoDC3000ΔhQ), we aim to understand the dynamics of wound entry and colonization in the model plant *Nicotiana benthamiana*. Luminescent tagged bacteria will be inoculated onto wounded leaf tissues, enabling observation of their movement and potential accumulation around wounded sites. This project incorporates chemical treatments and employs phytopathology, biochemistry, and a forward genetic approach to investigate the ability of bacterial leaf pathogens to colonize leaf tissue from wounded sites.
The outcomes of this study are highly pertinent to plant pathology, providing insights into the mechanisms governing bacterial pathogen entry and spread within plant tissues.

Recommended reading for the project

Misas-Villamil JC, Kolodziejek I, van der Hoorn RA. *Pseudomonas syringae* colonizes distant tissues in *Nicotiana benthamiana* through xylem vessels. Plant J. 2011:67(5):774–782. – DOI: 10.1111/j.1365-313X.2011.04632.x – PubMed: https://pubmed.ncbi.nlm.nih.gov/21554458/

Funding Form - BSPP Undergraduate Vacation Bursary Fund Application : Entry # 1863

Title of Project

Composition and field-relevance of biofilms in the wheat pathogen *Zymoseptoria tritici*

This project is going to be...

Experimental (lab/field based)

Full Name of Supervisor

Dr Helen Fones

Institution Department and Address

**University of Exeter, Biosciences, Stocker Road,
Exeter, Devon EX4 4QD
United Kingdom**

Full name of the day to day supervisor and/or arrangements for supervision

Dr Helen Fones will carry out the majority of day to day supervision, with input from Dr Graham Thomas (PDRF) where appropriate to provide extra support and training. Support and health and safety training will be provided by Ms Marina Albu, lab manager.
Additional microscopy training may be provided by members of the University of Exeter's Bioimaging team.

The student will benefit from:
An introduction to a working plant biology, plant pathology and microbiology lab. 1:1 training in microbiology techniques from either Helen Fones or members of her team; 1:1 training in microscopy from either Helen or specialist experimental officers based in our Bioimaging suite; the opportunity to gain experience in a range of advanced microscopy techniques such as

fluorescence microscopy, confocal microscopy and potentially others (light sheet, Raman, SEM). In addition the student will be imbedded in a wider lab environment comprising multiple research groups, and able to attend lab meetings and seminars given by members of this group and the wider department. They will be encouraged to attend 'Microbes and Disease' research theme events and meet a range of researchers at different career stages.

Date of Project Commencement

17/06/2024

Duration (weeks)

6

Brief Description of Project

Recent work in the Fones lab, carried out by our previous BSPP vacation bursary recipient, Tegan Tyzack, has demonstrated that the wheat pathogenic fungus, *Zymoseptoria tritici*, is able to form biofilms in vitro (Tyzack* et al., 2023, BioRXiv). Biofilms are formed when microbial cells are attached to a surface and embedded in an extracellular matrix (ECM) secreted which they secrete. They are often associated with stress tolerance, as the ECM can act as a 'shield' against stresses like drying out, high temperatures or microbicidal chemicals.
Tegan proved that biofilms form under low-nutrient conditions and are resistant to stresses such as drying, hydrogen peroxide and fungicides. These findings have opened a series of research questions: what is the composition of the extracellular matrix in which the biofilm cells are embedded, how it is produced, and how does it protect cells from stress? There is some evidence that biofilms form in planta, but are these 'true' biofilms with the same properties as seen in vitro? Do they have a protective function for *Z. tritici* in the field?
In this project, you will begin to answer some of those questions, using a combination of microscopy, fungal stress assays, and in planta experiments on wheat.
Firstly, you will use existing protocols to grow *Z. tritici* biofilms and non-biofilm blastospores. Using a range of fluorescent dyes, you will stain samples for possible ECM components such as glucans, proteins, or eDNA, and image the ECM using fluorescence and confocal microscopy. You will also measure potential ECM component concentrations in both biofilm and non-biofilm samples. Taken together, these experiments will begin to elucidate the chemical composition of the *Z. tritici* biofilm ECM.
Secondly, you will assess the ability of biofilms to withstand important field-associated stresses, both in vitro and in planta, using isolates of *Z. tritici* shown in preliminary data to produce biofilm-like structures in planta. You will assess fungal cell survival under UV, ROS and starvation stresses when growing in vitro as pelagic or biofilm cells vs in planta either as dispersed individuals or in a biofilm.
These experiments will help us begin to understand how and why *Z. tritici* produces biofilms.

Recommended reading for the project

Harding, M. W., Marques, L. L. R., Howard, R. J., & Olson, M. E. (2009). Can filamentous fungi form biofilms? Trends in Microbiology, 17(11), 475–480. https://doi.org/10.1016/J.TIM.2009.08.007
Michael W Harding, Lyriam Marques, Ronald J. Howard, & Merle Olson. (2010). Biofilm morphologies of plant pathogenic fungi. The Americas Journal of Plant Science and Biotechnology.
https://www.researchgate.net/publication/270890717_Biofilm_morphologies_of_plant_pathogenic_fungi
Tyzack, T. E., Hacker, C., Thomas, G., & Fones, H. N. (2023). Biofilm formation *in Zymoseptoria tritici*. BioRxiv, 2023.07.26.550639.
https://www.biorxiv.org/content/10.1101/2023.07.26.550639v1.abstract

Fones, H. N., Soanes, D., & Gurr, S. J. (2023). Epiphytic proliferation of *Zymoseptoria tritici* isolates on resistant wheat leaves. Fungal Genetics and Biology, 168, 103822. https://www.sciencedirect.com/science/article/pii/S1087184523000531
Peiqian, L., Xiaoming, P., Huifang, S., Jingxin, Z., Ning, H., & Birun, L. (2014). Biofilm formation by *Fusarium oxysporum* f. sp. *cucumerinum* and susceptibility to environmental stress. FEMS Microbiology Letters, 350(2), 138–145. https://doi.org/10.1111/1574-6968.12310

Funding Form - BSPP Undergraduate Vacation Bursary Fund Application : Entry # 1862

Title of Project

Can Potato Cyst Nematodes be effectively controlled by the introduction of resistant varieties?

This project is going to be...

Remote/virtual

Full Name of Supervisor

Adam Kleczkowski

Institution Department and Address

University of Strathclyde
Glasgow, Glasgow City G1 1XH
United Kingdom

Full name of the day to day supervisor and/or arrangements for supervision

The project will be jointly supervised by Prof. Adam Kleczkowski (University of Strathclyde) and Dr Jon Pickup (Scientific Advice for Scottish Agriculture, SASA). Although modelling will be the main aspect of the project, the student will benefit from a unique combination of supervision in mathematics and statistics (Kleczkowski) and PCN biology and policy (Pickup).
Kleczkowski will assume the day-to-day supervision, and the student will be given room and computer access at the University of Strathclyde in Glasgow. The student will visit SASA in Edinburgh at least monthly. The initial visit to the lab facilities will enable the student to learn about PCN and their detection. During subsequent visits, the student will discuss the progress with SASA scientists. The final visit will be devoted to a presentation of the results and a discussion of the impact on the industry.
Arrangements for remote work will be made for a suitable candidate.

Date of Project Commencement

01/07/2024

Duration (weeks)

10

Brief Description of Project

Production and export of seed potatoes are of large value to the UK economy (over £250m), and hence, the maintenance of our high health status is of paramount importance despite numerous threats by pests and pathogens. Potato cyst nematodes (PCN) are recognised as one of the most

damaging pests to GB potato growers. Planting resistant varieties is the most effective control method, capable of limiting pest population increases to below 1% compared to a susceptible host. However, in 1970s, resistance-breaking PCN populations of a separate species, *G. pallida*, emerged in the UK. Statutory testing data by SASA show that the area of land recorded as infested with *G. pallida* has been growing over the last 50 years. This is explained by the widespread cultivation of varieties susceptible to G. pallida, with resistant varieties only recently gaining market acceptance. This project will use the SASA-collected data on PCN species and epidemiological modelling to address two questions. Firstly, it will assess strategies to maximise the use of these varieties to control *G. pallida*. Secondly, under current legislation, seed potatoes can only be planted on PCN-free land, severely restricting the available area and requiring extensive testing. We will address the effect of possible relaxation of this restriction on the ability to control PCN. The project is suitable for students with mathematics or statistics backgrounds who will benefit from learning biology or for biology students with an interest in data analysis and modelling and provides a unique opportunity to work at cutting-edge interdisciplinary and policy-driven research.

Recommended reading for the project

PCN-related papers:
- https://www.pcnhub.ac.uk/sites/www.pcnhub.ac.uk/files/2023-02/pcn_introduction_factsheet_final.pdf - basic information on PCN and their impact on the industry
- J.A. Price, J. Pickup, M.A. Back, Potato Cyst Nematodes in Scotland and Their Impact from Farm to Fork, Plant Health Cases, doi:10.1079/planthealthcases.2023.0014.
- Two Plant Health Centre reports on PCN dynamics and modelling: https://www.planthealthcentre.scot/publications/pcn-working-group-final-report and https://www.planthealthcentre.scot/publications/modelling-spread-pcn-scotland

Modelling papers:
- Bailey DJ, Kleczkowski A, Gilligan CA. 2004 Epidemiological dynamics and the efficiency of biological control of soil-borne disease during consecutive epidemics in a controlled environment. New Phytologist 161, 569–575.
- Gibson GJ, Kleczkowski A, Gilligan CA. 2004 Bayesian analysis of botanical epidemics using stochastic compartmental models. Proceedings of the National Academy of Sciences 101, 12120–12124. (doi:10.1073/pnas.0400829101)
- Kleczkowski A, Gilligan CA. 2007 Parameter estimation and prediction for the course of a single epidemic outbreak of a plant disease. Journal of The Royal Society Interface 4, 865–877. (doi:10.1098/rsif.2007.1036)
- Kleczkowski A, Hoyle A, McMenemy P. 2019 One model to rule them all? Modelling approaches across OneHealth for human, animal and plant epidemics. Phil. Trans. R. Soc. B 374, 20180255. (doi:10.1098/rstb.2018.0255)

Funding Form - BSPP Undergraduate Vacation Bursary Fund Application : Entry # 1860

Title of Project

Studying the variation in virulence of the *Botryosphaeriaceae* using a novel pine seedling system

This project is going to be...

Experimental (lab/field based)

Full Name of Supervisor
David Read
Institution Department and Address
Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Lunnon Street Entrance, Hatfield Pretoria, Gauteng 0083 South Africa
Full name of the day to day supervisor and/or arrangements for supervision
The student will be under the main supervision of a research fellow (Dr David Read) and the co-supervision of a full professor (Prof Bernard Slippers) and an MSc student (Christiaan Grobler). The student will have weekly meetings with the team to ensure that they are on track with their project and to answer any technical and logistical questions. The student will have regular meetings with the team to discuss results and the implications of these results. The student will be part of an institution which consists of 36 professors, 8 research fellows, 13 post-doctoral fellows, 90 PhD candidates and 118 MSc candidates who all have interests and projects in the fields of plant pathology, ecology, molecular biology, entomology, mycology, virology, bacteriology, biochemistry and genetics who they can interact with and speak to should they have any questions or would like to expand their knowledge about any of the above topics. The institution has weekly presentations from post-doctoral researchers and seminars from PhD and MSc students who share updates on their research and research methodologies. The institution also has occasional special talks from international speakers and workshops presented by international professors and post-doctoral fellows. The student will be expected to join the weekly presentation by post-doctoral researchers as well as the seminars presented by the PhD and MSc students and will be encouraged to join the presentations and workshops offered by international guests should they be interested in the topics. A variety of journal clubs are also offered at the institution and the student will be encouraged to join a journal club that is of interest to them. The institution also has regular fieldtrips to commercial pine, eucalyptus and wattle plantations for disease monitoring, sample collections and lure-based trap installations as well as engage with industry partners. The student will be encouraged to participate in these fieldtrips which will allow them to visit these plantations and engage with members of the commercial forestry sector.
Date of Project Commencement
13/05/2024
Duration (weeks)
10
Brief Description of Project
Diplodia sapinea is a globally distributed endophyte of conifers hypothesized to originate in North America or Eurasia (Burgess et al., 2001; Aragonés et al., 2021). *Diplodia sapinea*, however, is also an opportunistic necrotrophic pathogen which infects stressed trees to cause Diplodia shoot dieback (Caballol et al., 2022). The transition from asymptomatic endophyte to pathogenic stages of *D. sapinea* appear to correlate to host stress which can be a result of wounding, drought or heat (Stanosz et al., 2001; Smith et al., 2002; Brodde et al., 2019). The extent of variation in virulence between different isolates remains poorly understood. We

therefore aim to better understand the variation observed in the virulence between different *D. sapinea* isolates using a novel pine seedling screening system.
The student will produce in vitro spores of *Diplodia sapinea* using a protocol described in Oostlander et al., (2023). The student will then quantify the spores using a microscope spore counter as well as a spectrophotometer. Following quantification, the student will infect three-week-old pine seedlings and induce heat stress and/or drought stress and then monitor the seedlings to make inferences about the virulence of different *D. sapinea* isolates in a collection that has been characterised using population genetics methods. The correlation between genetic diversity and variation in virulence will be explained.

Recommended reading for the project

Aragonés, A., Manzanos, T., Stanosz, G., Munck, I.A., Raposo, R., Elvira-Recuenco, M., Berbegal, M., Mesanza, N., Smith, D.R. and Simmons, M., et al. (2021), Comparison of Diplodia Tip Blight Pathogens in Spanish and North American Pine Ecosystems, Microorganisms 9.
Burgess, T., Wingfield, B.D. and Wingfield, M.J. (2001), Comparison of genotypic diversity in native and introduced populations of Sphaeropsis sapinea isolated from *Pinus radiata*, Mycological research 105: 1331–1339.
Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008). Dictionary of the Fungi (10th ed.). Wallingford: CABI. p. 100. ISBN 978-0-85199-826-8.
Oostlander, A.G., Brodde, L., Bargen, M. von, Leiterholt, M., Trautmann, D., Enderle, R., Elfstrand, M., Stenlid, J. and Fleißner, A. (2023), A Reliable and Simple Method for the Production of Viable Pycnidiospores of the Pine Pathogen *Diplodia sapinea* and a Spore-Based Infection Assay on Scots Pine, Plant disease 107: 3370–3377

Funding Form - BSPP Undergraduate Vacation Bursary Fund Application : Entry # 1859

Title of Project

Investigations into *Curreya pithyophila* and canker disease; a new health threat to Scots pine in Scotland

This project is going to be...

Experimental (lab/field based)

Full Name of Supervisor

Sarah Green

Institution Department and Address

Forest Research, Northern Research Station
Roslin, Midlothian EH259SY
United Kingdom

Full name of the day to day supervisor and/or arrangements for supervision

The main supervisor will be the senior pathologist, Dr Sarah Green, and any fieldwork will be conducted with Sarah and colleagues in a field working team. The student will have day-to-day laboratory supervision from the project team members including two pathologists, an entomologist and a mycologist/fungal taxonomist. The student will also meet regularly with the senior pathologist at Forest Research to assess and discuss project progress and outcomes.

Date of Project Commencement

24/06/2024

Duration (weeks)

10

Brief Description of Project

Since late 2022 there have been an increasing number of reports of canker and dieback symptoms on Scots pine (*Pinus sylvestris*) across Scotland. One of the primary causal agents was found to be a previously obscure stroma-forming fungus currently named *Curreya pithyophila*. Populations of this fungus in Scotland have been recently found to comprise two morphologically and genetically distinct 'types' which are currently being formally named as separate species. Both types of *C. pithyophila* form a black stroma which encircles young shoots, branches and main stems of Scots pine trees, most typically at branch junctions. Beneath the stroma are immature colonies of the native Scots pine woolly adelgid, *Pineus pini*, which feeds on the tree, initiating wounds. These wounds are then invaded by a longstanding fungal pathogen of pine, *Crumenulopsis sororia*, which causes the blackened, perennating cankers that expand, killing branches. So there are four causative agents involved in this outbreak of canker disease of Scots pine; two distinct fungal species currently known as *C. pithyophila* in association with *P. pini* as the primary agents of bark killing, and *C. sororia* as a secondary invader of the damaged bark. Although progress has been made in understanding the distribution of this disease, which is widespread now in Scotland, and identifying and describing the causal agents involved, more research is needed to understand the epidemiological factors driving infestations and their impact on native and commercial stands of Scots pine.

This project will be conducted within the broader framework of Scottish Plant Health Centre and Scottish Forestry funded projects focused on understanding the drivers of this emerging health threat to Scots pine. The specific objectives of this project are to; i) carry out lab based studies to investigate the environmental requirements for *C. piithyophila* spore release, spore germination and early development of stromatal infestations, ii) undertake inoculation studies to understand how/if *C. pithyophila* develops on young plants of Scots pine with and without the presence of *Pineus pini*, iii) assist with field surveys aimed at defining the impact of this canker disease on the health of Scots pine, iv) contribute to genetic analyses of *C. pithyophila* specimens to understand population diversity and existence of different mating types.

The student will be based within the pathology labs at Forest Research's Northern Research Station near Edinburgh and will learn a mix of traditional pathology and microscopy methods, and molecular techniques including DNA extraction, PCR and Sanger sequencing. The student will also assist with inoculation studies involving a collaboration between pathologists and entomologists. Additionally, the student will experience field work and learn survey methods used to estimate biotic health impacts on trees, including contributing to statistical analyses.

Recommended reading for the project

Green, S., Taylor, J.E., Stanisz-Migal, M., F. Tierney-Kitchener, Hendry, S.J., A'Hara, S., Lester, K., Davidson, M., Roehrig, L., Purser, E. 2024. Understanding a new health threat to Caledonian Scots pine (*Pinus sylvestris*). Project Final Report. PHC 2022/07. Scotland's Centre of Expertise for Plant Health (PHC).

Holm, L. 1967. Taxonomic Notes on Ascomycetes. Svensk Botanisk Tidscrift. BD 61, H. 4., 449-458.

McIntosh, C. 1915. *Cucurbitaria pithyophila*.Transactions of the Royal Scottish Arboricultural Society, Vol 29/30, 209-210.

Murray, J.S., Parry, W.H. 1969. The association of *Pineus pini* and *Cucurbitaria pithyophila* on Scots pine in northern Scotland. Scottish Forestry (1969), 8-13.

https://www.forestresearch.gov.uk/news/132823-a-new-old-health-threat-to-scots-pine/
https://www.planthealthcentre.scot/blog/iconic-caledonian-scots-pine-new-health-threat-and-its-implications-management

Funding Form - BSPP Undergraduate Vacation Bursary Fund Application : Entry # 1858

Title of Project

Characterizing the immune receptor network in sweet potato (*Ipomoea batatas*)

This project going to be...

Experimental (lab/field based)

Full Name of Supervisor

Lida Derevnina

Institution Department and Address

The crop science Centre, Department of plant Sciences, University of Cambridge, 93 Lawrence Weaver Rd,
Cambridge, Cambridgeshire CB3 0LE
United Kingdom

Full name of the day to day supervisor and/or arrangements for supervision

The student will join the plant-pathology 'supergroup' at the Crop Science Centre: a brand-new research institute and joint initiative between the University of Cambridge and the National Institute of Agricultural Botany that aims to accelerate the world's transition to sustainable agriculture.
The student will be supervised by Lida Derevnina (LD) and Unnati Sonawala (US; postdoctoral researcher in the group). This multidisciplinary and collaborative project will provide the student with a range of skills that will be greatly beneficial for their future careers and the experience of working in the collaborative environment that is commonplace in research.
The student will first be introduced to the study system and rationale for their project. In the lab, they will learn best laboratory practices by working closely with experienced members of LD group. They will be guided through the foundations of molecular cloning including transformation, plasmid extraction and sequencing followed by transient expression in Nicotiana benthamiana leaves. The student will be eventually encouraged to transition to semi-autonomous working days to develop their independence as a researcher. No previous experience is required.
The student will be well integrated into all social and academic aspects of the laboratory. They can expect daily contact with their supervisor(s) and intellectually stimulating discussions other members of the laboratory during weekly lab meetings. Additionally, the student will get the opportunity to participate and present at the biweekly plant pathology 'supergroup' lab meeting between LD and the Plant-parasite interactions group headed by Sebastian Eves-van den Akker. The student will be encouraged to participate in informal academic meetings to discuss their progress and in discussions on other projects during lab meetings. These formal and informal meetings will provide the supervisor(s) an opportunity to assess the student's progress and understanding of their project. At the end of the internship, the student will consolidate their knowledge, experience, and results into a formal presentation to the rest of the laboratory to showcase their critical assessment skills and progress.

Date of Project Commencement

17/06/2024

Duration (weeks)

10

Brief Description of Project

The plant immune system relies on nucleotide-binding domain and leucine-rich repeat-containing (NLR) proteins to respond to invading pathogens and activate immune responses. An emerging paradigm in NLR biology is that "sensor" NLR proteins are paired with "helper" NLR proteins for immune signalling. While this has been extensively studied in Solanaceous plants like tomato, this sensor-helper NLR network remains underexplored in the Convolvulaceae family, which includes the important food crop sweet potato *(Ipomoea batatas*). Fundamental understanding of how the sweet potato NLR network functions will have direct implications for resistance breeding strategies.
Root knot nematodes pose a significant threat to sweet potato production, causing substantial global yield losses. To identify the helper and sensor NLRs involved in immune response to root-knot nematodes, we have performed transcriptomic sequencing of the infected and non-infected portions of sweet potato roots. The student will participate in a programme of research aimed at elucidating the interaction between root-knot nematodes and sweet potato, by elucidating NLRs that are differentially expressed during this interaction. They will be involved in identifying, cloning, and characterizing sweet potato NLRs using the model organism *N. benthamiana*. The student will have the opportunity to: i) be exposed to a bioinformatics pipeline to mine NLR candidates from the recently generated transcriptomic data; ii) use modern cloning techniques to clone the candidate effectors into the relevant expression vectors; iii) and test their function using Agrobacterium-mediated expression in *N. benthamiana*. This multidisciplinary project involves computational and wet lab experiments that will be tailored to the interests and skills of the successful applicant, to maximise their contribution to the overall research programme.
Taken together, this project will provide an opportunity for immersive training in cutting edge plant-pathogen research to a new trainee, while uncovering the key immune players in sweet potato important in defence to root-knot nematodes.

Recommended reading for the project

Goh F-J, Huang C-Y, Derevnina L, Wu C-H (2023) NRC immune receptor networks show diversified hierarchical genetic architecture across plant lineages. BioRxiv https://doi.org/10.1101/2023.10.25.563953
Wu C-H, Derevnina L, Kamoun S (2018) Receptor networks underpin plant immunity. Science 10.1126/science.aat2623
Wu CH et al (2017) NLR network mediates immunity to diverse pathogens. PNAS https://doi.org/10.1073/pnas.1702041114

Funding Form - BSPP Undergraduate Vacation Bursary Fund Application : Entry # 1857

Title of Project

Development of culturing and resistance screening techniques for downy mildew (*Hyaloperonopora* sp) on wild rocket (*Diplotaxis tenuifolia*)

This project is going to be...

Experimental (lab/field based)

Full Name of Supervisor

Lauren Chappell

Institution Department and Address

Warwick Crop Centre, University of Warwick, Stratford Innovation Campus, Wellesbourne Warwick, Warwickshire CV35 9EF
United Kingdom

Full name of the day to day supervisor and/or arrangements for supervision

Dr Lauren Chappell with additional support by Prof John Clarkson
The student will be supervised day to day by Lauren Chappell. The student will be part of an established and engaging plant pathology lab group, with support via regular lab meetings as well individual project discussions.

Date of Project Commencement

01/07/2024

Duration (weeks)

10

Brief Description of Project

Downy mildew of wild rocket (*Diplotaxis tenuifolia*) caused by the oomycete *Hyaloperonopora* sp. has been identified by UK growers and breeders as an increasing problem to control. Primary symptoms include irregular yellow necrotic patches with white spores present on young leaves and cotyledons (Caruso et al., 2018), resulting in significant reductions in crop quality and major yield losses. Whilst it is thought that wild rocket *Hyaloperonopora* sp. are species specific and display no cross-infection with other brassica species (Coelho et al, 2022), little is understood about host resistance or pathogen population diversity. Growers have indicated that previously tolerant varieties are now displaying symptoms, which may indicate the presence of undescribed races in the UK. There is a therefore an urgent need to establish culturing protocols and plant infection assays to help industry identify new sources of resistance or races reliably.
This project will work closely with seed companies (Tozers Seeds, Elsoms Seeds, CN Seeds) and the British Leafy Salads Association (BLSA) to obtain isolates of *Hyaloperonospora* sp., and a range of rocket accessions. Initial work will focus on establishing an infection protocol to maintain and bulk the pathogen on rocket as it is an obligate biotroph and therefore cannot be cultured on artificial media. This is further challenged by the lack of long-term storage options, so further work will examine pathogen viability after freezing and other storage options. Once a robust infection protocol is established, the project will aim to develop resistance screening assays using susceptible and potentially tolerant / resistant wild rocket accessions provided by industry. Initial work will aim to identify resistant phenotypes and see if this concurs with

initial observations from growers and breeders. Developing infection protocols and resistance screening methodologies will provide underpinning methods for future projects and applications, particularly to investigate potential race structure which is well supported by industry given the challenges they are currently facing.
The final part of this project will focus on using molecular techniques to confirm species identity and potentially assess race structure. Initial work will look at optimising DNA extraction from *Hyaloperonopora* sp. spores and then carrying out barcoding gene (ITS,LSU, RpB2) PCR and sequencing to confirm *Hyaloperonopora* sp. species identity and diversity across several isolates.
This project is an excellent opportunity for a student to learn core pathology skills, including plant raising, pathogen culturing, DNA extraction and PCR amplification as well as sequence analysis and phylogenetics. The student will also have the opportunity to interact with industry partners and learn about their business and will help provide essential underpinning research to a commercial relevant problem for future industrial collaborations.

Recommended reading for the project

1. Coelho, P. S., Reis, J. M., Pereira, A. L., Vairinhos, A., Lopes, V., & Leitão, J. M. (2022). Downy mildew resistance and genetic variability in a wild rocket germplasm collection. Agronomy Journal, 114, 3083–3095. https://doi.org/10.1002/agj2.21190
2. Caruso, G.; Parrella, G.; Giorgini, M.; Nicoletti, R. (2018). Crop Systems, Quality and Protection of Diplotaxis tenuifolia. Agriculture, 8, 55. https://doi.org/10.3390/agriculture8040055

Funding Form - BSPP Undergraduate Vacation Bursary Fund Application : Entry # 1856

Title of Project

Investigating the emergence of new *Fusarium oxysporum* f. sp. *lactucae* races using long-read nanopore sequencing and comparative genomics

This project is going to be...

Experimental (lab/field based)
Remote/virtual

Full Name of Supervisor

Dr Helen Bates

Institution Department and Address

NIAB, 93 Lawrence Weaver Road
Cambridge, Cambridgeshire CB3 0LE
United Kingdom

Full name of the day to day supervisor and/or arrangements for supervision

Dr Helen Bates will supervise the student with additional bioinformatics support from Dr Jordan Price

Date of Project Commencement

15/07/2024

Duration (weeks)

10

Brief Description of Project

The soil-borne fungus *Fusarium oxysporum* (Fo) is a species complex (FOSC) of both pathogenic and non-pathogenic isolates with the former specific to different plant hosts each referred to as forma specialis (f. sp). For example, isolates that infect lettuce (*Lactuca sativa*) are Fo f.sp. lactucae (Fola). Pathogenic isolates cause wilt, crown, and root rot diseases across many plant species including key crops e.g. tomato, lettuce, onion, brassicas, cucurbits and banana (1). Some Fo f. sp are further divided into races that can infect previously resistant cultivars of the host plant.
Fusarium wilt of lettuce caused by Fola is a worldwide issue, causing crop losses of up to 50% (2). So far, four races have been described. Race 1 (Fola1) affects field grown lettuce in warmer areas such as Southern Europe, Asia, and the Americas. Races 2 and 3 are only present in Taiwan and Japan. Fusarium wilt of lettuce was unheard of in Northern Europe until new race 4 (Fola4) emerged in 2015 affecting glasshouse crops in the Netherlands. Fola4 has now spread to Belgium, the UK, and to open field production in Spain and Italy. Fola is therefore expanding its range northwards and is an increasing threat to lettuce production in Europe.
The FOSC genome can be divided into a set of conserved (core) chromosomes that carry essential housekeeping genes and are present across all Fo isolates, as well as highly variable (dispensable) accessory chromosomes that are enriched for transposons (3,4) and effector genes involved in Fo pathogenicity (5,6). Long-read sequencing is essential for resolving these regions due to their highly repetitive nature. Understanding the complement of effectors in these regions is essential to understand the genetic basis of host specificity in different Fo f. spp. and races. Work carried out at NIAB, in collaboration with Prof. John Clarkson at Warwick, has identified key differential genomic signatures between Fola1 and Fola4 isolates providing intriguing insights into their evolutionary origin. Also, recent work at Warwick characterising new Fola isolates from USA, France and Spain by gene-specific PCR, has identified several isolates that do not fit the usual profile of Fola1 or Fola4. These isolates also break resistance in lettuce lines previously resistant to Fola 1. This suggests the emergence of at least one new Fola race.
The aim of this 10 week project is to shed light on the evolution of this new Fola race by characterising genomes of the representative isolates using nanopore sequencing and comparative genomics against previously characterised Fola1 and Fola4 isolates. The work will be based at NIAB, Cambridge (some remote working possible). A visit to Warwick for a hands-on experience in inoculating lettuce plants with Fo will also be possible. The student will learn a wide range of skills including culturing of Fo, DNA extraction, nanopore sequencing, bioinformatic analyses, and plant fungus bioassays.

Attach the recommended reading for the project

1. Edel-Hermann and Lecomte, 2019 doi:10.1094/PHYTO-08-18-0320-RVW
2. Gilardi et al., 2017 doi:10.1111/ppa.126163 3.
3. Ma et al., 2010 doi:10.1038/nature08850
4. Yang et al., 2020 doi:10.1094/PHYTO-03-20-0069-IA
5. Armitage et al., 2018 DOI:10.1038/s41598-018-30335-7,
6. Van Dam et al., 2017 doi:10.1038/s41598-017-07995-y

Funding Form - BSPP Undergraduate Vacation Bursary Fund Application : Entry # 1854

Title of Project

Characterising the Role of Ubiquitin in Immune Response Activation

This project is going to be...

Experimental (lab/field based)

Full Name of Supervisor

Beatriz Orosa

Institution Department and Address

The King's Buildings
Edinburgh, UK EH9 3BF
United Kingdom

Full name of the day to day supervisor and/or arrangements for supervision

During the student's initial two weeks in the lab, I will personally supervise them to provide comprehensive training on laboratory protocols and techniques. Following this introductory period, they will transition to working closely with my third-year PhD student, Karolina Brzezinska, who will guide the student through ongoing projects. We will hold weekly meetings to review progress, address any challenges, and discuss potential solutions. Additionally, our lab conducts weekly meetings where all students share their weekly progress in a supportive environment. This practice fosters presentation skills development, encourages critical thinking, and promotes teamwork by facilitating discussions, questions, and suggestions among peers.

Date of Project Commencement

03/06/2024

Duration (weeks)

8

Brief Description of Project

Plant diseases pose significant threats to UK agriculture and horticulture, leading to substantial food and financial losses and endangering global food security. Preventing crop diseases caused by pathogens and pests is crucial for the success of the UK agri-industry sector, with fungal plant pathogens being particularly concerning due to their significant yield losses. In response to pathogen attacks, plants initiate immune responses, but adapted pathogens can suppress these responses using effector proteins, leading to severe crop damage. Ubiquitination, a key mechanism for immune activation and defence response regulation, involves attaching the small polypeptide ubiquitin to substrate proteins. This process offers rapid and reversible modulation of protein function, regulating stress response intensity and amplitude to enhance pathogen resistance. However, the comprehensive role of the ubiquitome in regulating plant immune responses remains poorly understood.
This undergraduate project aims to elucidate how ubiquitination regulates plant immune responses by studying Arabidopsis mutants for different immune Ubiquitin E3 ligases identified in our immune ubiquitome. By characterising our lab's collection of ubiquitin mutants, we aim to determine the role of immune Ubiquitin E3 ligases in plant defence responses. This research has the potential to advance our understanding of plant immunity and posttranslational

modification fields.
The internship offers interdisciplinary training covering genetics, molecular biology, pathology, and various techniques such as gene expression analysis, cloning, protein detection, and microscopy. Participants will work within a large, multidisciplinary team focusing on posttranslational modifications at the Institute of Plant Sciences at the University of Edinburgh.
The main tasks across the eight weeks are:
· Infect Arabidopsis ubiquitin mutants with different pathogens.
· Assess the different levels of resistance/susceptibility of the different mutants to each pathogen. Characterize the effect of pathogenesis by measuring the lesions, pathogen biomass, and evaluation of plant cell death by tissue stain.
· Validate the activation of immune genes in infected Arabidopsis plants by QPCR.
· Assess the accumulation of ubiquitin in response to the infection by purification of ubiquitinated targets and western blot.

Recommended reading for the project

1. FAO 2017 (http://www.fao.org/3/a-i6583e.pdf); 2. Zhang, X and Cai, X. Climate change impacts on global agricultural land availability Vol 6(2011); 3. Savary, S et al. Nat Ecol Evol 3(3): 430-439 (2019); 4. https://ahdb.org.uk ; 5. Oerke, EC. Journal of Agricultural Science 144, 31 -43 (2006); 6. Whelan H. G et al. Plant pathology 47; 397-406 (1997); 8. Zipfel C and Robatzek S. Plant Physiol154(2):551-4(2010); 9. Orosa, B. et al. PLoS Genetics13, e1006540(2017); 10. Skelly, M et al. Elife 8. pii: e47005 (2019); 11. Spoel et al. Cell137(5):860-72 (2009); 12. Orosa, B. et al.

Funding Form - BSPP Undergraduate Vacation Bursary Fund Application : Entry # 1851

Title of Project

Under attack by a cereal killer: enhancing wheat's resilience to rust infection

This project is going to be...

Experimental (lab/field based)

Full Name of Supervisor

Professor Diane G.O. Saunders

Institution Department and Address

John Innes Centre
Norwich, Norfolk NR4 7UH
United Kingdom

Full name of the day to day supervisor and/or arrangements for supervision

The student will be supported day-to-day by two assigned mentors (Dr Swathy Puthanvila-Surendrababu and Ms Sarah Bailey) that will closely guide and train the student and work together with the student through all aspects of the project.

Date of Project Commencement

01/07/2024

Duration (weeks)

10

Brief Description of Project

Introduction
Wheat is a major staple cereal for 40 percent of the world's population. It is the most widely cultivated crop in the world, providing 20 percent of the daily human dietary calorie and protein requirements. However, this principal food crop is under constant threat from rapidly evolving fungal diseases, with rust diseases a great concern. The global production of wheat has increased significantly over the last few decades and so has demand, especially in developing countries which harvest 50 percent of global wheat production annually. However, there is an urgent need to sustainably increase wheat production and productivity to meet the ever-increasing global demand for food. Combating wheat diseases such as wheat rusts, among other biotic and abiotic factors will undoubtedly contribute to reducing wheat yield losses, which will ultimately boost food security worldwide.
Project Aim
The aim of this project is to investigate the role of wheat genes shown in the Saunders Lab to be linked to supporting yellow rust disease susceptibility. Notably, yellow rust (YR) disease is caused by the biotrophic fungal pathogen *Puccinia striiformis* f. sp. *tritici* (Pst), a wheat destroyer and major threat to wheat production worldwide. Previous work (based on RNA sequencing) in the Saunders Laboratory identified candidate wheat genes that can be disrupted to restrict Pst infection; EMS mutant Kronos durum wheat plants where each of these genes has been disrupted displayed significantly reduced wheat yellow rust infection. However, to ensure these gene disruptions can be used in widely grown wheat varieties worldwide requires the gene disruptions to be tested in a broader set of wheat variety backgrounds. To this aim, in this project the student will evaluate CRISPR wheat mutants that have been generated in three wheat varieties that are suited for growth in East Africa and act as key varieties in breeding programmes for the region. Once the CRISPR wheat mutants have been assessed for yellow rust susceptibility, this will provide critical supporting information for their progression into field trials in East Africa.
Your Role
In this project, you will combine genetic, molecular and plant pathology approaches to assess the effect of the gene disruptions in wheat on rust infection. This will include cultivating and genotyping wheat CRISPR mutant lines and their respective wildtype plants; performing rust infection assays to phenotypically evaluate disease progression in the mutants relative to the wildtype; gene expression analysis (by quantitative real time PCR).
This project will provide a unique opportunity for training in a broad array of techniques and you will also be embedded in a large, multi-disciplinary research group led by Professor Diane Saunders at the JIC.

Recommended reading for the project

Corredor-Moreno P., et al., *Saunders D.G.O. (2023) Temporally coordinated expression of nuclear genes encoding chloroplast proteins in wheat promotes *Puccinia striiformis* f. sp. *tritici* infection. Communications Biology, 5: 853. [https://doi.org/10.1038/s42003-022-03780-4]
Corredor-Moreno P., Minter F., Davey P.E., Wegel E., Kular B., Brett P., Lewis C.M., Morgan Y.M.L., Macías Pérez L.A., Korolev A.V., Hill L., *Saunders D.G.O. (2021) The branched-chain amino acid aminotransferase TaBCAT1 modulates amino acid metabolism and positively regulates wheat rust susceptibility. The Plant Cell, 5: 1728-1747. [https://doi.org/10.1093/plcell/koab049]
Minter F., *Saunders D.G.O. (2023) Safeguarding wheat yields from cereal fungal invaders in the postgenomic era. Current Opinion in Microbiology, 73. [https://doi.org/10.1016/j.mib.2023.102310]

Funding Form - BSPP Undergraduate Vacation Bursary Fund Application : Entry # 1850

Title of Project

Function of two tandemly duplicated immune receptor genes in disease resistance and its temperature sensitivity

This project going to be...

Experimental (lab/field based)

Full Name of Supervisor

Dr Henrik Stotz

Institution Department and Address

College Lane
Hatfield, Hertfordshire AL10 9AB
United Kingdom

Full name of the day to day supervisor and/or arrangements for supervision

Supervisor: Dr. Henrik Stotz
assistance is available by two PhD students and lab managers

Date of Project Commencement

03/06/2024

Duration (weeks)

10

Brief Description of Project

Within the context of climate change, temperature-resilient disease resistance mechanisms are expected to be of value to crop breeders and farmers. SNC1 is a central regulator of temperature-sensitive plant immunity in *Arabidopsis thaliana*. We have identified a BrSNC1 ortholog in *Brassica rapa* that is located next to the FocBr1 gene for resistance against *Fusarium oxysporum*. TILLING mutants were identified in both genes, and preliminary tests suggest that a Brsnc1 mutant allele provides temperature resilient resistance against *Leptosphaeria maculans*, whereas a focBr1 mutant allele was hypersusceptible to this fungal pathogen.

For this Undergraduate Vacation Bursary, the student will be testing advanced backcross generations to confirm the above-mentioned observations. A segregating population of the focBr1 line exists. Approximately 50 progeny plants will be tested at 20oC and 25oC in controlled environment cabinets for their susceptibility to *L. maculans*. Cotyledons will be used for KASP marker genotyping, whereas true leaves will be used to assess disease severity after spot inoculations.

Out-segregant wild-type and Brsnc1 mutants will be used to compare their susceptibility to *L. maculans*. Infected leaf samples will be harvested and analysed for pathogen biomass using established qPCR methodologies.

It is anticipated that successful completion of this project will contribute to a publication in an international peer-refereed journal.

Recommended reading for the project

Shimizu, M. et al. Map-based cloning of a candidate gene conferring Fusarium yellows resistance in *Brassica oleracea*. Theor Appl Genet 128, 119-130 (2015). https://doi.org:10.1007/s00122-014-2416-6
Miyaji, N. et al. Development of a new DNA marker for Fusarium yellows resistance *in Brassica rapa* vegetables. Plants (Basel) 10, 1082 (2021). https://doi.org:10.3390/plants10061082
Noel, K. et al. Influence of elevated temperatures on resistance against phoma stem canker in oilseed rape. Front. Plant Sci. 13, 785804 (2022).
Stotz, H. U. et al. *Leptosphaeria maculans* isolates with variations in AvrLm1 and AvrLm4 effector genes induce differences in defence responses but not in resistance phenotypes in cultivars carrying the Rlm7 gene. Pest Manag Sci (2023). https://doi.org:10.1002/ps.7432

Funding Form - BSPP Undergraduate Vacation Bursary Fund Application : Entry # 1833

Title of Project

Manipulating resource flow between parasites and mutualists of plants

This project is going to be...

Experimental (lab/field based)

Full Name of Supervisor

Dr Chris Bell

Institution Department and Address

Centre for Plant Sciences
Leeds, W Yorkshire LS29JT
United Kingdom

Full name of the day to day supervisor and/or arrangements for supervision

Dr Chris A Bell - supervise full time
Dr Catherine J Lilley (postdoctoral researcher) - supervise full time
Dr Mirela Coke (technician) - supervise for technical aspects of the project

Date of Project Commencement

03/06/2024

Duration (weeks)

6

Brief Description of Project

Plant-parasitic nematodes and mutualistic arbuscular mycorrhizal fungi are very different organisms, however they both rely upon their host plant for the entirety of their resource intake. Their ability to acquire resources from their hosts will dictate their success as well as impact plant growth and yield. i.e. success of the parasite will dramatically reduce plant vigour, whilst success of the mutualist may lead to enhanced yields. Lack of understanding of the mechanisms underpinning host resource transport to different symbionts hinders our progress

towards creating plants that can differentiate between friend and foe and allocate their resources accordingly. This project within the Plant Nematology Group in Leeds will determine the impact of knocking out genes that may underlie either specific or common resource flow to plant-parasitic nematodes and mycorrhizal fungi. The aim of this project is to determine whether we can selectively restrict resource flow to detrimental symbionts whilst maintaining flow to those symbionts that impart a benefit to the plant. The student will be fully integrated into the Plant Nematology Group so that they get a true taste of the research environment. They will gain experience in a range of molecular techniques, such as CRISPR/Cas9 in crop plants, qPCR to measure gene expression and tissue culture maintenance of explants. The student will work with both animals and plants, providing a wide range of transferrable skills and if desired, there is the opportunity to utilise bioinformatics to determine novel target genes. The work package can be tailored to the skills/wishes of the student and the outputs will aid future efforts to maximise food production.

Recommended reading for the project

Bell, C.A et al. 2022. Disruption of carbon for nutrient exchange between potato and arbuscular mycorrhizal fungi enhanced cyst nematode fitness and host pest tolerance. New Phytologist. 234; p269-279.
Bell, C.A. et al 2024. Phytophagy impacts the quality and quantity of plant carbon resources acquired by mutualistic arbuscular mycorrhizal fungi. Nature Communications. Accepted. Will email to applicants.

Funding Form - BSPP Undergraduate Vacation Bursary Fund Application : Entry # 1832

Title of Project

Unleashing AI-powered predictions for plant-pathogen interactions

This project is going to be...

Experimental (lab/field based)

Full Name of Supervisor

Dr. Brian Mooney

Institution Department and Address

Department of Biology, South Parks Road, University of Oxford
Oxford, Oxfordshire OX1 3RB
United Kingdom

Full name of the day to day supervisor and/or arrangements for supervision

Dr. Brian Mooney is a postdoctoral researcher in the laboratory of Prof. Renier van der Hoorn at the University of Oxford and will be the day to day supervisor for this project. I have significant experience supervising multiple student projects including an MSc in Biochemistry student at Oxford in 2023-2024.
This proposal is submitted with the full agreement of Prof. Renier van der Hoorn to host the project in the Plant Chemetics laboratory and the student will have the opportunity to participate in activities with the wider research group (e.g. lab group meetings / journal clubs).

Date of Project Commencement

08/07/2024

Duration (weeks)

6

Brief Description of Project

Plant diseases cause massive crop losses annually with devastating effects for global food security and local economies. Developing a deeper understanding of how plants resist pathogens could lead to the development of more resilient crop varieties in the future. The colonisation of plant tissues by pathogens typically begins in the extracellular space (apoplast). During infection, plants secrete an array of defence-related enzymes to the apoplast to resist pathogen attack. In response, pathogens can release 'effector' proteins that may neutralise host defences, e.g. by forming inhibitory protein complexes with hydrolase enzymes. These apoplastic plant-pathogen interactions are crucial in determining the progression of plant disease but only a limited number have been characterised in detail.
In 2021, advances in artificial intelligence culminated in the release of AlphaFold, a groundbreaking protein-structure prediction technology that has dramatically improved our capacity to predict molecular events in biology. Ongoing research in the Plant Chemetics laboratory focuses on using the updated Alphafold-Multimer platform to predict putative apoplastic protein complexes that may be functionally relevant for plant immunity. For example, one recent study used this approach to identify novel interactions between defence-related hydrolase enzymes from tomato and secreted proteins released by major tomato pathogens (including *Pseudomonas syringae*, *Botrytis cinerea* and *Cladosporium fulvum*) (Homma et al., 2023). The AI-predicted cross-kingdom protein complexes were subsequently validated experimentally using a range of biochemical techniques, including activity-based protein profiling (ABPP).
Considering the success of this approach, we have since applied Alphafold-Multimer to explore other plant pathosystems. Most recently, we have produced a comprehensive list of highly promising candidate apoplastic interactions between the model plant Nicotiana benthamiana and the model pathogen *Pseudomonas syringae*. The proposed project seeks to validate and characterise these putative interactions in the laboratory using molecular cloning, agroinfiltration, protein expression, protein purification and ABPP among other techniques.
The student undertaking this project will gain exposure to the cutting edge of plant pathology research, with the opportunity to learn essential laboratory techniques and develop greater understanding of plant immunity and the utility of AI-based predictions in biology. Outside of the laboratory, the student will also benefit from access to other activities within the research group, including the opportunity to participate in lab group meetings and journal club sessions. Finally, the student undertaking this project will gain valuable experience working at the University of Oxford which is globally-renowned for the quality of its education and impactful research.

Recommended reading for the project

AlphaFold-Multimer predicts cross-kingdom interactions at the plant-pathogen interface
https://www.nature.com/articles/s41467-023-41721-9
Apoplastic Proteases: Powerful Weapons against Pathogen Infection in Plants
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7748006/
The Increasing Impact of Activity-Based Protein Profiling in Plant Science
https://pubmed.ncbi.nlm.nih.gov/26872839/