Entry #1183: Investigation of the effectiveness of fungicides for control of phoma stem canker pathogens of oilseed rape

Title of Project
Investigation of the effectiveness of fungicides for control of phoma stem canker pathogens of oilseed rape
This project going to be...
Experimental (lab/field)
Full Name of Supervisor
Dr Chinthani Karandeni Dewage
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Position held
Research Fellow
Full name of the day to day supervisor and/or arrangements for supervision
Dr Chinthani Karandeni Dewage
Date of Project Commencement
13/06/2022
Duration (weeks)
8
Brief Description of Project
Background: Phoma stem canker (PSC) is a major disease problem on oilseed rape (Brassica napus) in the UK, causing losses worth more than £80M p.a. The disease is caused by two closely related species, Leptosphaeria maculans (Lm) and L. biglobosa (Lb), which co-exist on their host. Lm is generally considered more damaging than Lb, because Lm is often associated with damaging stem base cankers and Lb is often associated with less damaging upper stem lesions. However, studies show that Lb can also cause substantial yield losses in areas where Lb is present as the predominant pathogen. Although, both Lm and Lb are present in UK, there has been little work on Lb with no control strategies targeted on Lb. There have been previous studies reporting that Lb is less sensitive to some

azole fungicides that are commonly used for the control of phoma stem canker (Eckert et al. 2010; Huang et al. 2011). Investigation of fungicide-sensitivity in other fungal pathogens showed that mutations in the sterol 14α-demethylase (CYP51) gene is the main cause of development of fungicide insensitivity/resistance against azole fungicides (Cools & Fraaije, 2008; Carter et al., 2014). Although differences in sensitivity to flusilazole amongst Lm isolates have been observed, no mutations were detected in the Lm CYP51 gene (Huang et al., 2011). There have been no major structural differences identified between the predicted Lm and Lm CYP51 protein models (Sewell et al. 2017). Therefore, this project aims to investigate the invitro and in planta CYP51 gene expression differences between Lm and Lb as a contributing factor for the fungicide insensitivity.

Methodology: Student will be provided with RNA samples extracted from cotyledons of B. napus inoculated with L. maculans or L. biglobosa. The sampling has been done at two different time points (3 and 12 days after inoculation). The work involves cDNA synthesis and RT PCR with specific primers to detect the in planta CYP51 gene expression of these two pathogens. Mycelia samples have also been collected from invitro culture of the two pathogens. These samples will be used to investigate the invitro CYP51 gene expression from L. maculans and L. biglobosa. Comparison of gene expression between the two pathogens both in planta and in vitro can be used to improve our knowledge about the differences between these two pathogens in their insensitivity to azole fungicides.

This project will enable the student to understand key principles of plant pathology and to gain skills in molecular biology techniques including, RNA extraction, PCR, gene-expression analysis, etc. The student will work as part of a research group interacting with other PhD, MSc research students working on various aspects of plant pathology (host resistance, pathogen population studies, inter-species interactions, etc.), that will be beneficial for the students development.

Attach the recommended reading for the project

Eckert, M. R., Rossall, S., Selley, A. & Fitt, B. D. L. Effects of fungicides on in vitro spore germination and mycelial growth of the phytopathogens Leptosphaeria maculans and L. biglobosa (Phoma stem canker of oilseed rape). Pest Manag. Sci. 66, 396–405 (2010).

Huang, Y. J. et al. Effects of fungicide on growth of Leptosphaeria maculans and L. biglobosa in relation to development of phoma stem canker on oilseed rape (Brassica napus). Plant Pathol. 60, 607–620 (2011).

Carter, H. E. et al. Alterations in the predicted regulatory and coding regions of the sterol 14α-demethylase gene (CYP51) confer decreased azole sensitivity in the oilseed rape pathogen Pyrenopeziza brassicae. Mol. Plant Pathol. 15, 513–522 (2014).

Cools, H. J. & Fraaije, B. A. Are azole fungicides losing ground against Septoria wheat disease? Resistance mechanisms in Mycosphaerella graminicola. Pest Manag. Sci. 64, 681–684 (2008).

Sewell, Thomas R., et al. "Azole sensitivity in Leptosphaeria pathogens of oilseed rape: the role of lanosterol 14α-demethylase." Scientific Reports 7.1 (2017): 1-12.

Entry #1181: Genome Assembly and Prediction of Metabolic and Functional Trait Profiles of Pectobacterium Species Isolated from Soft Rotting Potato Tubers

Title of Project
Genome Assembly and Prediction of Metabolic and Functional Trait Profiles of Pectobacterium Species Isolated from Soft Rotting Potato Tubers
This project going to be...
Remote/virtual
Full Name of Supervisor
Dr Ciara Keating
Institution Department and Address
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Position held
Post Doctoral Research Associate
Full name of the day to day supervisor and/or arrangements for supervision
Dr Keating will co-supervise and deliver training to the student with Dr Ijaz (Reader in Bioinformatic Engineering: http://userweb.eng.gla.ac.uk/umer.ijaz/). They will be integrated into the Environmental Omics Lab and the cross-disciplinary Blackleg Hub team (https://www.blackleghub.ac.uk). Training and daily meetings will be held on Teams/Zoom which will cover plans for the week and training on techniques. The student will attend weekly project meetings where they will show Powerpoint slides of their progress. - Skills: Dr Keating has co-supervised 13+ computational MSc projects with Dr Ijaz from 2020-2022.
Date of Project Commencement
09/05/2022
Duration (weeks)
10
Brief Description of Project

Background
The potato is one of the most popular vegetables in the UK. The potato grower industry suffers losses in excess of £50M per year from potato blackleg disease. Blackleg disease is a soft rot disease whereby pathogenic bacteria (Pectobacterium spp) produce enzymes (cellulases and pectinases for example) that allow them to degrade the plant cell walls. This capability is actually quite interesting in terms of biotechnology. Food, plant and paper mill waste contain high volumes of cellulose and lignocellulose. A biotechnological process – termed anaerobic digestion can be applied to degrade these wastes and convert them to renewable energy in the form of methane gas. However, the initial break-down of cellulose is often a rate-limiting step in the process and leads to poor methane yields. Yet, Pectobacterium breaks down plant cell walls relatively easily. In this studentship, we will analyse the genomes of Pectobacterium species, with a particular focus on the metabolic functions and their contribution to biogeochemical cycling (e.g. the carbon cycle) using state of the art bioinformatics software.

Objectives
Therefore the aims and objectives of this studentship are to:
• Assemble the genomes of 20+ novel Pectobacterium isolates with demonstrated ability to initiate soft rot infection in five potato varieties from multiple agricultural field sites (Week 1 – Week 3).
• Annotate the genomes to recover metabolic function in particular focusing on major biogeochemical pathways such as Carbon, Nitrogen and Sulfur, and other nutrient cycles (Week 4 – Week 6).
• Compare the genomes (SNPs, genes) to existing genomes of Pectobacterium species available in public repositories such as NCBI (Week 7 – Week 10)

Supervision and Benefit to the Student
This will be a remote computational research project. The student should have basic computational skills. I will co-supervise and deliver training to the student with Dr Ijaz (Reader in Bioinformatic Engineering: http://userweb.eng.gla.ac.uk/umer.ijaz/). They will be integrated into the Environmental Omics Lab and the cross-disciplinary Blackleg Hub team (https://www.blackleghub.ac.uk). The student will also attend the wider grant consortium meetings (held on a bi-monthly basis) where updates from all the involved institutes are shown. Importantly, these meetings have all the staff from the project from across the UK and key industry contacts such as potato growers, SASA, and other stakeholders. We anticipate that these findings will form part of a short research or perspective article citing that Pectobacterium may be adaptable for biotechnological solutions.

Attach the recommended reading for the project

Literature related to blackleg disease and Pectobacterium:

Wolf, J.M., Acuña, I., Boer, S.H.D., Brurberg, M.B., Cahill, G., Charkowski, A.O., Coutinho, T., Davey, T., Dees, M.W., Degefu, Y. and Dupuis, B., 2021. Diseases caused by Pectobacterium and Dickeya species around the world. In Plant diseases caused by Dickeya and Pectobacterium species (pp. 215-261). Springer, Cham.

Toth, I.K., Barny, M.A., Brurberg, M.B., Condemine, G., Czajkowski, R., Elphinstone, J.G., Helias, V., Johnson, S.B., Moleleki, L.N., Pirhonen, M. and Rossmann, S., 2021. Pectobacterium and Dickeya: environment to disease development. In Plant Diseases

Caused by Dickeya and Pectobacterium Species (pp. 39-84). Springer, Cham.

Skelsey, P., Humphris, S.N., Campbell, E.J. and Toth, I.K., 2018. Threat of establishment of non-indigenous potato blackleg and tuber soft rot pathogens in Great Britain under climate change. PLoS One, 13(10), p.e0205711.

Literature related to computational methods that will be used in this project:

Stratakos, A.C., Ijaz, U.Z., Ward, P., Linton, M., Kelly, C., Pinkerton, L., Scates, P., McBride, J., Pet, I., Criste, A. and Stef, D., 2020. In vitro and in vivo characterisation of Listeria monocytogenes outbreak isolates. Food Control, 107, p.106784.
Dingle, K.E., Didelot, X., Quan, T.P., Eyre, D.W., Stoesser, N., Marwick, C.A., Coia, J., Brown, D., Buchanan, S., Ijaz, U.Z. and Goswami, C., 2019. A role for tetracycline selection in recent evolution of agriculture-associated Clostridium difficile PCR ribotype 078. MBio, 10(2), pp.e02790-18.

Page, A.J., Cummins, C.A., Hunt, M., Wong, V.K., Reuter, S., Holden, M.T., Fookes, M., Falush, D., Keane, J.A. and Parkhill, J., 2015. Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics, 31(22), pp.3691-3693.

Brynildsrud, O., Bohlin, J., Scheffer, L. and Eldholm, V., 2016. Rapid scoring of genes in microbial pan-genome-wide association studies with Scoary. Genome biology, 17(1), pp.1-9.

Zhou, Z., Tran, P.Q., Breister, A.M., Liu, Y., Kieft, K., Cowley, E.S., Karaoz, U. and Anantharaman, K., 2022. METABOLIC: high-throughput profiling of microbial genomes for functional traits, metabolism, biogeochemistry, and community-scale functional networks. Microbiome, 10(1), pp.1-22.

Li, X., Ma, Y., Liang, S., Tian, Y., Yin, S., Xie, S. and Xie, H., 2018. Comparative genomics of 84 Pectobacterium genomes reveals the variations related to a pathogenic lifestyle. BMC genomics, 19(1), pp.1-22.

Entry #1179: Ash dieback: analysis and modelling of the Realising Ash Potential field trials

Title of Project
Ash dieback: analysis and modelling of the Realising Ash Potential field trials
This project going to be...
Experimental (lab/field), remote/virtual, Computational, on site
Full Name of Supervisor
Prof. Adam Kleczkowski
Institution Department and Address
Dept. Mathematics and Statistics, University of Strathclyde, 26 Richmond Street Glasgow, Glasgow G1 1XH

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Telephone

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Email

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Position held

Professor

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

The project will be jointly supervised by Prof. A Kleczkowski (Strathclyde) and Dr J Clark (Future Trees Trust). The data analysis and modelling will be carried out in Glasgow, Scotland, but there is a possibility of carrying out the work entirely remotely. The student will also have an opportunity to travel to Oxfordshire to take part in collecting the 2022 data. The student will be given appropriate training by Dr Clark and the Future Trees Trust; transport and accommodation will be covered from the project budget.

Date of Project Commencement

15/06/2022

Duration (weeks)

10

Brief Description of Project

Ash dieback, Hymenoscyphus fraxineus, is a fungal disease that has been inflicting devastating impacts on the UK landscapes and biodiversity since its first detection in 2012; it is expected to have a £15b impact to the UK economy. It is now widespread in Europe, with up to 85% mortality rates recorded in plantations and 69% in woodlands. It is estimated that a large proportion of ash trees in the UK are already affected. As there is no promising treatment or prevention measure, the best hope for the long-term future of the UK's ash trees lies in identifying tolerant or resistant trees for breeding new generations.

This project combines unique field data sets collected during in the Realising Ash Potential trials and supplied by Dr Clark from the Future Trees Trust, and Prof. Kleczkowski modelling approaches. The student will also benefit from collaboration with a PhD student jointly supervised by Kleczkowski and Dr Cavers from the Centre of Ecology and Hydrology (CEH). Data on 4,212 trees collected for different provenances, consisting of budburst, senescence and infection levels assessed yearly since 2013, will be analysed statistically to uncover a relationship between tree provenance and disease resistance. An epidemiological model, based on a survival (Cox) model, will be constructed and fitted to data using appropriate statistical approaches. Depending on student interests, a Bayesian approach will be considered, allowing estimation of survival probabilities. An extension to an epidemiological, Susceptible-Infected-Removed, model will also be considered.

The student will also have an opportunity to travel to Oxfordhshire to take part in collecting the 2022 data and to subsequently analyse them and incorporate into the model. The student will be given appropriate training and support in travelling to the site.

The project suits a student of quantitative subjects, or a Biology student who wants to expand their portfolio by including advanced epidemiological modelling. The student will learn about ash biology and Ash Dieback impact, including the ability to identify disease in the field and the principles of field trials. They will also study basic spatial statistics and disease modelling, before progressing to using R to construct a model. Time permitting, the student will also explore how climate change is impacting spread of disease by combining epidemiological data with weather records.

The student will have the opportunity to present their work to members of CEH and Future Trees. They will also benefit from participation in a large NERC Treescape grant NE/V019988/1 " Learning to adapt to an uncertain future: linking genes, trees, people and processes for more resilient treescapes (newLEAF)" with Kleczkowski leading one of the work packages. The student will participate in regular project meetings and will present the results of the project for the consortium.

Attach the recommended reading for the project

Literature (modelling):

• 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)

Literature (ash dieback):

• Forest Research: Pest and disease resources, Ash dieback (Hymenoscyphus fraxineus) https://www.forestresearch.gov.uk/tools-and-resources/fthr/pest-and-disease-resources/ash-dieback-hymenoscyphus-fraxineus/ [Accessed 01/03/2022]
• McKinney LV, Nielsen LR, Collinge DB, Thomsen IM, Hansen JK, Kjær ED. 2014 The ash dieback crisis: genetic variation in resistance can prove a long-term solution. Plant Pathology 63, 485–499. (doi:10.1111/ppa.12196)
• Landolt J, Gross A, Holdenrieder O, Pautasso M. 2016 Ash dieback due to Hymenoscyphus fraxineus: what can be learnt from evolutionary ecology? Plant Pathology 65, 1056–1070. (doi:10.1111/ppa.12539)
• Plumb WJ, Coker TLR, Stocks JJ, Woodcock P, Quine CP, Nemesio-Gorriz M, Douglas GC, Kelly LJ, Buggs RJA. 2020 The viability of a breeding programme for ash in the British Isles in the face of ash dieback. PLANTS, PEOPLE, PLANET 2, 29–40. (doi:10.1002/ppp3.10060)
• Mitchell RJ et al. 2014 Ash dieback in the UK: A review of the ecological and conservation implications and potential management options. Biological Conservation 175, 95–109. (doi:10.1016/j.biocon.2014.04.019)
• Whittet R, Cottrell J, Cavers S, Pecurul M, Ennos R. 2016 Supplying trees in an era of environmental uncertainty: Identifying challenges faced by the forest nursery sector in Great Britain. Land Use Policy 58, 415–426. (doi:10.1016/j.landusepol.2016.07.027)

Entry #1178: Disrupting NLR networks: the case of plant-parasitic nematodes.

Title of Project
Disrupting NLR networks: the case of plant-parasitic nematodes.
This project going to be...
Experimental (lab/field)
Full Name of Supervisor
Lida Derevnina
Institution Department and Address
93 Lawrence Weaver Road Cambridge, Cambridgeshire CB30LE United Kingdom Map It
Telephone
01223337150
Email
LD645@cam.ac.uk
Position held
Head of Group
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 have the benefit of joint supervision by experts in each respective part of the project: Lida Derevnina (LD), and members of her newly established lab, will be the primary supervisor and mentor for aspects related to plant immunity; Sebastian Eves-van den Akker (SEvdA) and members of his lab will supervise aspects related to effector discovery. 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. They will then be guided through computational experiments and challenged to design a pipeline to aid in effector discovery in collaboration with SEvdA. In the lab, they will learn laboratory best practices by working closely with experienced members of the LD and SEvdA groups. They will be guided through the foundations of molecular cloning, transformation, plasmid extraction, sequencing etc, before transitioning 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 regular meetings with their supervisor(s) and daily contact with experienced members of the laboratory. 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 supervisors 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

18/07/2022

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. In the Solanaceae, a major phylogenetic clade of NLRs form a complex immunoreceptor network in which multiple helper NLRs are required by a much larger number of sensor NLRs to mediate immunity against diverse pathogens. Many of these helper NLRs are highly, or exclusively, expressed in roots—implicating them in immunity against root infecting pathogens, such as potato cyst nematodes. As such, helper NLRs are likely targets of pathogen derived molecules, known as effectors, as a means of overcoming plant immune responses. Thus, studying the extent to which effectors suppress helper NLRs offers an opportunity to understand the evolution and robustness of the NLR network.

A pilot study involving a fraction of nematode effector diversity has already revealed that plant parasitic nematodes secrete effectors to target helper NLRs expressed in solanaceous roots. Using the model Solanaceae plant, Nicotiana benthamiana, as a study system, we previously tested 26 nematode effectors for immunosuppression function. Of those 26 effectors, ~ 10% were shown to suppress NLR network activity. We hypothesise many unknown effectors also function to suppress NLR network activity. Extending this study across the greater nematode effectorome is of paramount importance and will lead to greater insights into the mechanisms parasitic nematodes use to perturb immune receptor networks, and thus potential interventions to manage this pest in agriculture.

The student will participate in a programme of research aimed at dissecting the immune-supressing functions of potato cyst nematode effectors, by working to identify, clone, and screen nematode effectors for helper NLR-suppressing ability in N. benthamiana. The student will have the opportunity to: i) develop a bioinformatics pipeline to mine effector candidates from the genome of potato cyst nematodes, based on recent advances in gland-cell sequencing technology; ii) use synthetic biology techniques to clone the candidate effectors into the relevant expression vectors; iii) and test their activity for suppression of helper NLRs 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, and uncover details of the fascinating and agronomically important co-evolutionary arms race that occurs between potato cyst nematodes and their solanaceous plant hosts.
Attach the recommended reading for the project
Wu et al 2017 NLR network mediates immunity to diverse plant pathogens PNAS 114(30) https://doi.org/10.1073/pnas.1702041114 Wu, Derevnina et al 2018 Receptor networks underpin plant immunity Science 360(6395) DOI: 10.1126/science.aat2623 Derevnina et al 2021 Plant pathogens convergently evolved to counteract redundant nodes of an NLR immune receptor network PLoS Biology https://doi.org/10.1371/journal.pbio.3001136 Eves-van den Akker, Sebastian. "Plant–nematode interactions." Current opinion in plant biology 62 (2021): 102035. https://doi.org/10.1016/j.pbi.2021.102035

Entry #1173: Establishing a Cereal-Pseudomonas Pathosystem for Comparative Phytopathology Research

Title of Project
Establishing a Cereal-Pseudomonas Pathosystem for Comparative Phytopathology Research
This project going to be...
Experimental (lab/field)
Full Name of Supervisor
Philip Carella
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Position held
Group Leader
Full name of the day to day supervisor and/or arrangements for supervision
Dr. Phil Carella (group leader) will routinely meet with the student and advise on experimental design and implementation. Dr. Khong-Sam Chia (postdoc) will support the

student in the lab and with infection assays, phenotyping, and bacterial growth determination. Additional training on bacterial transformations and genetic manipulation will be done with Dr. Phil Carella.
Date of Project Commencement
03/07/2022
Duration (weeks)
8
Brief Description of Project
Pseudomonas syringae (Psy) is a widespread bacterial pathogen that causes significant damage to plants worldwide. While a vast amount of molecular pathology research has focused on it's interactions with model dicots like Arabidopsis and tomato, several agricultural and ecological surveys have identified Psy isolates with expansive host ranges that include monocots like cereal crops. To begin to identify and understand mechanisms of virulence that are important for cereal infection, the student will screen a diverse set of 75 Psy isolates for their ability to cause disease in the model cereal Hordeum vulgare. Upon isolating a reliable virulent strain, they will then assess virulence in additional monocot crops like wheat and maize. Despite the time constraints the student will also contribute to resource development by generating type 3 secretion system (T3SS)-deficient mutants (hrcC) unable to utilize effector proteins and/or mutants in common bacterial phytoxins. Time permitting, this will enable them to assess the contribution of core virulence factors to cereal infection. Thus far our preliminary analyses using a set of strains has identified differential capacities of Psy isolates to promote disease in distantly-related plants. Integrating monocot crops into this framework will enable further comparative analyses that will shed light on generalized-vs-specific virulence principles within the Pseudomonas syringae species complex.
Attach the recommended reading for the project
Evolution of Pseudomonas syringae: https://pubmed.ncbi.nlm.nih.gov/30606234/

Entry #1169: Study of proteolysis of plant immune receptors

Title of Project
Study of proteolysis of plant immune receptors
This project going to be...
Experimental (lab/field)
Full Name of Supervisor
Dr. Mariana Schuster
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Position held

Post-doctoral researcher

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

Dr. Mariana Schuster

Date of Project Commencement

13/06/2022

Duration (weeks)

6

Brief Description of Project

Background:

Immune receptors are arguably the most important proteins of the plant immune system as these are responsible for initiating the immune response. Immune receptors located at the plasma membrane constitute the frontline of pathogen recognition. Due to their crucial function, the homeostasis, activities, and subcellular dynamics of these receptors are subjected to tight regulation. Understanding the function and regulation of immune receptors therefore promises to reveal new opportunities for crop protection.
Proteolytic cleavage of immune receptors is emerging as a necessary regulatory process of immune receptor function. Through proteomics data mining, we have generated a list of receptor candidates that undergo proteolysis in vivo. The next step is to validate these candidates in planta.

Aim:

Investigate proteolysis of immune receptors of the model plant Nicotiana benthamiana.

Methods and tools:

1. Generation of Agroinfiltration constructs for the transient expression of immune receptors in Nicotiana benthamiana
2. Preparation of plant extracts for biochemical analysis
3. Visualization of receptor proteolysis via western blot
4. Challenge of receptor proteolysis with protease inhibitors

Attach the recommended reading for the project

Kong L, Rodrigues B, Kim JH, He P, Shan L. (2021) More than an on-and-off switch: Post-translational modifications of plant pattern recognition receptor complexes. Curr Opin Plant Biol. Oct;63:102051. DOI: 10.1016/j.pbi.2021.102051.

Entry #1166: Identification of the subcellular localisation of fungal pathogen effectors

Title of Project
Identification of the subcellular localisation of fungal pathogen effectors
This project going to be...
Experimental (lab/field) remote/virtual
Full Name of Supervisor
Martin Darino
Institution Department and Address
Department of Biointeractions and Crop Protection, Rothamsted Research, West Common Harpenden, Hertfordshire AL5 2JQ United Kingdom Map It
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Position held
Post-Doctoral Research Scientist
Full name of the day to day supervisor and/or arrangements for supervision
Dr. Martin Darino will be the main supervisor taking care of the day to day supervision. He possesses deep experience in fungal transformation, molecular and cell biology techniques developed it earlier (Darino et al., 2021). Dr. Darino will help the student in the cloning of the mitochondria marker and evaluation in N. benthamiana. He will provide the Fusarium strains expressing FgSSP41-mCherry and support the student in the wheat infections and confocal microscopy. Dr. Dan Smith will support the student with the evaluation of different bioinformatics approaches to identify new Fusarium effectors with subcellular localization. He is bioinformatic scientist in the institute with extensive experience in bioinformatic applied to study different plant – pathogen interactions. Finally, every 15 days it will be a meeting to discuss the results with Dr. Kim Hammond-Kosack, deputy head of the department.
Date of Project Commencement
04/07/2022
Duration (weeks)

10

Brief Description of Project

Fusarium graminearum (Fg) is a devastating fungal disease of wheat and a serious health hazard due to the contamination of the crop by harmful toxins. Fg is the predominant causal agent of Fusarium Head Blight (FHB). The pathogen secretes proteinaceous effectors that suppress the plant's immune response to promote infection. After secretion some effectors exert their functions in the apoplast, whilst others can be translocated to the cytoplasm. Once inside the host cell, effectors can target different subcellular structures such as the nucleus, endoplasmic reticulum, Golgi and organelles. For example, one Fg effector (FgSSP41) is now thought to localise to the mitochondria of Nicotiana benthamiana leaves. The identification of the subcellular localisation of other FgSSP effectors is essential to identify the range of potential effector functions and their intended host targets. Using this new knowledge will help to devise novel, durable, crop disease control strategies. The aim of this project is to validate the mitochondria localisation of FgSSP41 and identify the intracellular localisation of other early in planta expressed FgSSP effectors previously revealed using different bioinformatic approaches. To verify or refute mitochondrial localisation of FgSSP41, the student will clone a mitochondria marker and evaluate co-localisation with FgSSP41 in N. benthamiana using Agrobacterium infiltration and confocal microscopy. Then, already available Fg strains expressing FgSSP41 fused to mCherry will be used to infect wheat, the native host, to explore subcellular localisation. Finally, different bioinformatic approaches including Localizer (https://localizer.csiro.au/), EffectorP (https://effectorp.csiro.au/) and SignalP 6.0 (https://services.healthtech.dtu.dk/service.php?SignalP) on the Fg genome / Fg pan-genome will be used to identify FgSSP effectors with sequence signatures that suggest localisation in the nucleus, chloroplasts and/or mitochondria.
This project is laboratory based and is suitable for students looking to develop a career in pathogens infecting plants, animals, or humans. In addition, the bioinformatic analysis can be done remotely in case of new COVID restrictions. The student will gain experience in different molecular biology techniques like primer design, PCR, cloning and bacteria transformation; Agrobacterium transformation and N. benthamiana infiltrations; aseptic microbiology techniques, Fusarium infection of wheat plants, confocal microscopy and learn about different bioinformatic approaches to study effectors. The project will be based at Rothamsted Research in Harpenden, Hertfordshire, a world leading institute in crop sciences. In addition, this project will allow the student to improve their presentation and problem solving skills as well as provide the possibility to establish new connections for your future career development in academia or in industry.

Attach the recommended reading for the project

Literature about plant pathogen effectors:

Figueroa et al., 2021 . Tactics of host manipulation by intracellular effectors from plant pathogenic fungi. Curr. Opin. in Plant Biol. 62:102054. https://doi.org/10.1016/j.pbi.2021.102054.

Tzelepis et al., 2021 . Plant mitochondria and chloroplasts are targeted by the Rhizoctonia solani RsCRP1 effector. Biochem Biophys Res Commun. https://doi: 10.1016/j.bbrc.2021.01.019.

Paper about the techniques to be used in this project:

Darino et al., 2020. Ustilago maydis effector Jsi1 interacts with Topless corepressor, hijacking plant jasmonate/ethylene signaling. New Phytologist, 229 (6), 3393-3407. https://nph.onlinelibrary.wiley.com/doi/full/10.1111/nph.17116

Wood et al., 2020 . Functional evaluation of a homologue of plant rapid alkalinisation factor (RALF) peptides in Fusarium graminearum. Fungal Biol., 124(9), 753-765. https://doi.org/10.1016/j.funbio.2020.05.001.

Jiang et al., 2020 . An orphan protein of Fusarium graminearum modulates host immunity by mediating proteasomal degradation of TaSnRK1α. Nat. Commun. 11: 4382. https://doi.org/10.1038/s41467-020-18240-y.

Papers about the bioinformatics tools to be used in the project:

Sperschneider et al., 2017. LOCALIZER: subcellular localization prediction of both plant and effector proteins in the plant cell. Sci Rep 7: 44598. https://doi.org/10.1038/srep44598.

Sperschneider et al., 2016. EffectorP: predicting fungal effector proteins from secretomes using machine learning. New Phytol 210(2):743-61. https://doi: 10.1111/nph.13794.

Teufel et al., 2022. SignalP 6.0 predicts all five types of signal peptides using protein language models. Nat. Biotechnol. https://doi.org/10.1038/s41587-021-01156-3

A review about the most important fungal pathogens:

Dean et al., 2012. The top 10 fungal pathogens in molecular plant pathology. Molecular Plant Pathology 13: 414–430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

Entry #1159: Is the function of a key effector protein in the fungal plant pathogen *Z. tritici* specific to pathogenic lifestyles?

Title of Project
Is the function of a key effector protein in the fungal plant pathogen Z. tritici specific to pathogenic lifestyles?
This project going to be...
Experimental (lab/field)
Full Name of Supervisor
Luca Steel
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United Kingdom
Map It

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Position held

PhD student

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

Luca Steel, with additional support from Jason Rudd and Carlos Bayon.

Date of Project Commencement

04/07/2022

Duration (weeks)

10

Brief Description of Project

Background: Zymoseptoria tritici is a major fungal pathogen of wheat, causing a disease called Septoria Tritici Blotch. This leads to reduced yields and financial losses. Z. tritici secretes an important effector protein, Zt3LysM, during infection of wheat. This allows Z. tritici to mask its own chitin (a surface cell wall component) to evade detection, and to avoid its hyphae being degraded by enzymes secreted by wheat leaves. We have a fungal strain which has been engineered to lack this gene (deleted - "ΔZt3LysM") which has strongly reduced virulence on wheat leaves. We have also found that many other fungal species with wide-ranging lifestyles contain proteins with similar sequences. But are they able to perform the same function as Zt3LysM?

This project will: complete the first stages of an investigation into whether proteins with similar sequences in other fungal species are functional homologues of Zt3LysM. The student will analyse fungal genomes to design and generate constructs for use in a functional complementation assay. To do this, fungal species (with various lifestyles including non-pathogens) of interest containing proteins with similar sequences to Zt3LysM will be identified. Six of these will be chosen for further analysis, to cover at least: one known orthologous protein from a plant pathogen to act as a positive control; two additional plant pathogens (one wheat pathogen and one non-wheat pathogen); one saprophytic species; and one endophytic species. This will ensure a range of fungal lifestyles are investigated. Genes encoding the selected proteins will be cloned and prepared for transformation into the Z. tritici ΔZt3LysM strain. In a related PhD project, these strains will then be tested to see if they can infect wheat plants thereby testing if the genes from these other fungi are "real" LysM effectors. This will involve a literature review, as well as training in PCR and primer design, gel electrophoresis, Gibson assembly and cloning. This will enhance our understanding of a key effector protein and whether its function is specific to pathogenic lifestyles and potentially reveal cryptic (currently unknown) interactions of other fungi with plants. This will also establish a Z. tritici based method for examining the function of proteins in fungal species which are less amenable to laboratory investigation.

Other aims: Through this project, the student will learn and use key laboratory skills which are used widely across disciplines, such as cloning, PCRs, gel electrophoresis, micropipetting, microbial culture, and sterile technique. The student will also gain an understanding of plant pathology and molecular biology. Finally, the student will experience life as a scientist - honing skills in recording, analysing and reporting results, attending internal meetings and seminars, and contributing work to a paper for publication.
Attach the recommended reading for the project
Dean, R., Van Kan, J. A., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., & Foster, G. D. (2012). The Top 10 fungal pathogens in molecular plant pathology. Molecular plant pathology, 13(4), 422. https://doi.org/10.1111/j.1364-3703.2011.00783.x Orton, E. S., Deller, S., & Brown, J. K. (2011). Mycosphaerella graminicola: from genomics to disease control. Molecular plant pathology, 12(5), 413–424. https://doi.org/10.1111/j.1364-3703.2010.00688.x Tian, H, MacKenzie, CI, Rodriguez-Moreno, L, et al (2021). Three LysM effectors of Zymoseptoria tritici collectively disarm chitin-triggered plant immunity. Molecular Plant Pathology, 22(6), 683– 693. https://doi.org/10.1111/mpp.13055

Entry #1158: Investigating the role of cell wall components in response to *Fusarium graminearum* infection in *Arabidopsis thaliana*

Title of Project
Investigating the role of cell wall components in response to *Fusarium graminearum* infection in *Arabidopsis thaliana*
This project going to be...
Experimental (lab/field)
Full Name of Supervisor
Professor Kim Hammond-Kosack
Institution Department and Address
Biointeractions and Crop Protection Department, Centenary Building, West Common Harpenden, Herts AL5 2JQ United Kingdom Map It
Telephone
01582763240
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kim.hammond-kosack@rothamsted.ac.uk

Position held
Principal Research Scientist
Full name of the day to day supervisor and/or arrangements for supervision
Victoria Armer, PhD student (2nd Year). Plus weekly meetings jointly with Victoria Armer and Kim Hammond-Kosack for wider project guidance.
Date of Project Commencement
27/06/2022
Duration (weeks)
8
Brief Description of Project
Fusarium graminearum is the causal agent of Fusarium Head Blight (FHB) on small grain cereals such as wheat, oats and barley. Utilising a variety of cell wall degrading enzymes (CWDEs) and toxins, this ascomycete fungus colonises host plant tissue causing extensive cell death, contamination of grain with toxins and reduces crop yields. Host plants respond to infection by reinforcing cell walls with components such as pectin and lignin. Many cell wall mutants are available in the model plant species Arabidopsis thaliana, which will be utilised in this project to screen for cell wall components that are instrumental to host defence during F. graminearum infection. In this project the following activities will be pursued: • Utilisation of the plant model Arabidopsis thaliana due to range of cell wall mutants available for testing for infection assays. • Functionally test individual cell wall components for their role(s) in the host plant response to Fusarium graminearum infection. • Assess disease severity in leaf and floral infection assays, developing skills in imaging, ImageJ processing software and/or python programming. • Training in statistical analysis in R and presentation of data. • Handling of a pathogen, including various aseptic techniques, growth in vitro, spore preparation and inoculations in planta. • Development of molecular biology skills through fungal burden analysis, involving gDNA extraction and qPCR. • Bioinformatics search for wheat orthologues and expression patterns during F. graminearum infection using open-source tools including Uniprot, Ensembl and the wheat expression browser. • Involvement in student activities at Rothamsted, including seminars, journal clubs and lab meetings. Opportunity in late summer to present research project/ key findings to entire Wheat Pathogenomics team. • If covid-restrictions are in place, a back-up project will expand on the bioinformatics component exploring wheat orthologues to cell wall components known to be involved in defence in other pathosystems.
Attach the recommended reading for the project

Review on Arabidopsis vs wheat infection biology:
Brewer and Hammond-Kosack (2015). Host to a stranger: Arabidopsis and Fusarium Ear Blight. Trends in Plant Science. 20(10): 651-663. doi: 10.1016/j.tplants.2015.06.011

Fusarium + Arabidopsis research:
Urban et al. (2002). Arabidopsis is susceptible to the cereal ear blight fungal pathogens Fusarium graminearum and Fusarium culmorum. The Plant Journal. 32(6): 961-973. doi: 10.1046/j.1365-313X.2002.01480.x

Arabidopsis cell wall composition and disease resistance:
Molina et al. (2021). Arabidopsis cell wall composition determines disease resistance specificity and fitness. PNAS. 118(5): e2010143118. doi: 10.1073/pnas.2010243118

Entry #1156: Investigation of diversity in environmental *Pseudomonas syringae* in *Prunus* spp

Title of Project
Investigation of diversity in environmental *Pseudomonas syringae* in *Prunus* spp
This project going to be...
Experimental (lab/field)
Full Name of Supervisor
Ziyue Zeng
Institution Department and Address
NIAB, 93 Lawrence Weaver Road Cambridge, Cambridgeshire CB3 0LE United Kingdom Map It
Telephone
07729892836
Email
ziyue.zeng@niab.com
Position held
Postdoctoral Researcher
Full name of the day to day supervisor and/or arrangements for supervision
Ziyue Zeng The students will receive day-to-day supervision and will have regular meetings with their supervisor and mentor. The first meeting in week 1 will ensure the student is familiar with the work environment, is

provided with project details, and complete all necessary induction and health and safety training/documentation.

Date of Project Commencement

02/05/2022

Duration (weeks)

10

Brief Description of Project

Background information:
Pseudomonas syringae (P. syringae) is a globally important plant pathogen that has been reported to cause diseases on over 180 plant species, cherry included. P. syringae can infect and cause canker symptoms on the stems, branches, twigs, fruits, leaves, buds and blossoms of cherry trees, resulting in damage to trees, reduction of yields and low-quality or unsalable fruit. Despite many advances in disease control, P. syringae remains of great concern to the security of cherry production. Importantly there are many different strains (called pathovars) of Pseudomonas that can cause disease on cherry and very few cultivars have resistance to all known pathovars. The overall aim of our project is to provide fundamental insights into how new strains of pathogens emerge and evolve on potential hosts using P. syringae as an exemplar. This work will lead to improved design of control methods and new precision in risk monitoring tools.

Project objective:
To understand P. syringae population similarity and stability over distance, time and environment on and in the leaves of Prunus spp.

Experimental plan:
• Environmental bacterial isolation from defined sampling sites working within a team
• Bacterial strain purification, identification and characterisation
• DNA extraction and genome sequencing of key isolates of Pseudomonas
• Analysis of bacterial lineage persistence and diversity

Expected learning outcomes:
The student will learn microbiology, molecular biology and basic web-based bioinformatics skills. Specifically:
• Environmental bacterial strain isolation and identification
• PCR and agarose gel electrophoresis
• Bacterial pathogenicity test
• DNA extraction, DNA library preparation and sequencing
• Growth and care of cherry plants in glasshouse

Attach the recommended reading for the project

https://doi.org/10.1111/ppa.13189
https://doi.org/10.1111/nph.15182
https://doi.org/10.1111/ppa.12834

Entry #1146: Wheat phenotyping during root infection by take-all and functional characterization of wheat defense genes

Title of Project
Wheat phenotyping during root infection by take-all and functional characterization of wheat defense genes
This project going to be...
Experimental (lab/field)
Full Name of Supervisor
Wanxin Chen
Institution Department and Address
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Telephone
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Position held
Postdoctoral Researcher
Full name of the day to day supervisor and/or arrangements for supervision
Wanxin Chen and Javier Palma-Guerrero
Date of Project Commencement
06/06/2022
Duration (weeks)
10
Brief Description of Project
Take-all disease is the most important disease of wheat roots. It is caused by the fungus Gaeumannomyces tritici, that infects wheat roots and damages the vasculature tissue of the plants, therefore affecting water and nutrient uptake. Diseased plants show reduced yield and grain quality. In normal years, the disease causes between 5 to 20% yield losses, but it can be over 50% on years of high disease levels. The damage to the root system can also have a strong environmental impact, by the nitrate leaching from the soil as a result of the reduced crop's capacity to uptake nitrogen. So far, no genetic resistance to take-all has been found among modern wheat cultivars, and despite the importance of the disease, very little is known about the molecular mechanisms of virulence in the fungus. In this project, the

selected student will phenotype adult wheat plants infected by different strains of the take-all fungus to compare the effect of these strains on the adult plant health. Different aboveground and belowground measurements, indicators of plant health, will be taken at the adult stage. In addition, we will perform functional characterization of candidate wheat genes with a potential role in protecting wheat roots from take-all disease by using Virus Induced Gene Silencing (VIGS) and Virus Induced Overexpression (VOX). We have recently established this methodology for wheat roots. Candidate wheat genes have been identified by RNAseq during infection by a beneficial fungus that protects wheat roots from take-all disease by activating plant defenses. Plants with the candidate genes transiently silenced by VIGS or transiently overexpressed by VOX will be phenotyped at the seedling stage in presence and absence of take-all to study the role of the genes in the protection against take-all. The roots will be assessed for disease levels and the expression levels of the target genes will be evaluated by RT-qPCR.

Rothamsted Research is one of the longest running agricultural research institutions in the world. It counts with a multidisciplinary environment of scientists covering different expertise and working together with the goal to improve sustainable agriculture production. The student working in this project will learn to perform phenotypic aboveground and belowground measurements on adult wheat plants, VIGS, VOX, root disease assessments at seedling stage, RNA extraction and RT-qPCR. In addition, along the project the student will also learn to do fungal inoculations, work in sterile conditions, microbiology techniques, quantitative disease assessments, microscopy, experimental design, data analysis, results interpretation, presentation skills and report writing for the BSPP Newsletter.

Attach the recommended reading for the project

Palma-Guerrero et al 2021.Take-All Disease: New Insights into an Important Wheat Root Pathogen. Trends in Plant Science 26 number 8.

Bouton et al 2018. Foxtail mosaic virus: A Viral Vector for Protein Expression in Cereals. Plant Physiology Vol. 177.

Lee et al 2015. Virus induced gene silencing (VIGS) for functional analysis of wheat genes involved in Zymoseptoria tritici susceptibility and resistance. Fungal Genetics and Biology 79.

Bennypaul et al 2012. Virus-induced gene silencing (VIGS) of genes expressed in root, leaf, and meiotic tissues of wheat. Funct Integr Genomics 12:143–156.

Entry #1145: Boosting plant disease resistance through RNAi hairpin introduction

Title of Project

Boosting plant disease resistance through RNAi hairpin introduction

This project going to be...

Experimental (lab/field)

Full Name of Supervisor

Helen Cockerton

Institution Department and Address

Stacey Building
Canterbury, Kent CT2 7NJ
United Kingdom
Map It

Telephone

01227762349

Email

H.cockerton@kent.ac.uk

Position held

Research Fellow

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

Dr Helen Cockerton will be the primary supervisor of the student. Dr Cockerton has recently taken up a position as a research fellow, as part of her new role she is setting up plant pathology facilities at the University of Kent. The student will be supported day to day by Dr Cockerton. The student will become incorporated in all group lab meetings and will be encourage to attend seminars, they will also interact with other members of the Kent Fungal Group laboratory.

Date of Project Commencement

20/06/2022

Duration (weeks)

10

Brief Description of Project

Plant pathogens can cause extensive damage to crops, and if left untreated, epidemics can lead to complete crop destruction. New biotechnologies such as Host Induced Gene Silencing (HIGS) can be used to provide an environmentally friendly strategy for disease control. Here we ask whether RNAi hair pins can boost baseline plant immunity or whether targeted hair pins are required to generate disease resistant plants.

Published work has shown that exogenous application of random siRNA can create disease resistant plants through the upregulation of pathogen triggered immunity. Preliminary data suggests that “internally generated” siRNA produced through the introduction of a hairpin can also upregulate a plants base line immunity, irrespective of the hair pin target. Here we will study whether the introduction of an off-target RNAi hairpin can generate disease resistant plants.

Over the course of the placement a summer student will transform the model plant Arabidopsis thaliana to contain premade constructs. Four transformation lines will be produced, these will target 1) a control gene that is not present in the plant or the pathogen 2) a pathogenicity gene present in a fungal pathogen 3) a transcription factor present in a fungal pathogen 4) an empty hairpin vector. The student will also conduct disease assays on

pre-existing A. thaliana HIGS lines to assess disease resistance status to both Botrytis cinerea and Verticillium dahliae. This placement will generate resources to study the impact of “off-target” HIGS on a plants base line immunity. Ultimately, the project will shed light on whether there is a secondary mechanism of resistance created by the use of RNA hairpins to reveal an underexploited mechanism of disease resistance.
Attach the recommended reading for the project
Song, Yin, and Bart PHJ Thomma. "Host-induced gene silencing compromises Verticillium wilt in tomato and Arabidopsis." Molecular plant pathology 19.1 (2018): 77-89. Fan, Rong, et al. "Vegetative compatibility groups partition variation in the virulence of Verticillium dahliae on strawberry." PLoS One 13.2 (2018): e0191824. Fusaro, Adriana F., et al. "RNA interference-inducing hairpin RNAs in plants act through the viral defence pathway." EMBO reports 7.11 (2006): 1168-1175. Choudhary, Swati, et al. "A double-stranded-RNA response program important for RNA interference efficiency." Molecular and cellular biology 27.11 (2007): 3995-4005.

Entry #1144: Biofilm formation in *Zymoseptoria tritici*

Title of Project
Biofilm formation in *Zymoseptoria tritici*
This project going to be...
Experimental (lab/field)
Full Name of Supervisor
Helen Fones (Eyles)
Institution Department and Address
Biosciences Exeter, Streatham Campus, DEVON EX4 4QD United Kingdom Map It
Telephone
07830534580
Email
hnfones@gmail.com
Position held
UKRI Future Leaders Fellowship
Full name of the day to day supervisor and/or arrangements for supervision

The day to day supervisor will be me (Dr Helen Fones).
Support will also be given to the student by my Post-Doctoral Research Fellow, Dr Graham Thomas, and my Research Technician, Dr Andrea Kovacs-Simon. Between us, we will be able to ensure that the student can access help and advice at any time in the working day/week.

Date of Project Commencement

01/06/2022

Duration (weeks)

10

Brief Description of Project

This project will follow the work of a previous project student, looking into biofilm formation on leaf surfaces by the wheat pathogen, Zymoseptoria tritici. This fungus causes very significant yield losses and fungicide costs for temperate grown wheat. Previous findings in the Fones lab indicate that Zt spends extended periods on the leaf surface prior to and during infection. This epiphytic growth is subject to abiotic stresses such as UV exposure, drying, fungicide application and varying temperatures.

Biofilms are primarily known in bacteria and yeasts. They are formed when these microbes attach to a substrate and produce an extracellular matrix (ECM) in which the cells are embedded. Biofilm production leads to improved stress tolerance and can be importance in anti-microbial resistance.

Zt has a yeast-like growth form. While infection is dependent upon the switch to hyphal growth on the leaf surface, there can be extensive yeast-like proliferation on the leaf surface as well, and these yeast-like cells are able to produce infective hyphae. Previous work suggests that Zt yeast-like cells are able to produce ECM and may form a structure similar to other known biofilms.

In this project, you will explore the factors that promote biofilm formation and the characteristics of the biofilm, including morphology, cell type, and stress resistance. You will also carry out preliminary experiments to determine whether Zt isolates differ in biofilm formation capacity and whether this correlates to the success of infection under abiotic stress.

Attach the recommended reading for the project

Fones, H., & Gurr, S. (2015). The impact of Septoria tritici Blotch disease on wheat: An EU perspective. Fungal genetics and biology, 79, 3-7.

Haueisen, J., Möller, M., Eschenbrenner, C. J., Grandaubert, J., Seybold, H., Adamiak, H., & Stukenbrock, E. H. (2017). Extremely flexible infection programs in a fungal plant pathogen. bioRxiv, 229997.

Fones, H. N., Eyles, C. J., Kay, W., Cowper, J., & Gurr, S. J. (2017). A role for random, humidity-dependent epiphytic growth prior to invasion of wheat by Zymoseptoria tritici. Fungal Genetics and Biology, 106, 51-60.

Ten Cate, J. M., Klis, F. M., Pereira-Cenci, T., Crielaard, W., & De Groot, P. W. J. (2009).

Molecular and cellular mechanisms that lead to Candida biofilm formation. Journal of dental research, 88(2), 105-115.

Abdallah, M., Benoliel, C., Drider, D., Dhulster, P., & Chihib, N. E. (2014). Biofilm formation and persistence on abiotic surfaces in the context of food and medical environments. Archives of microbiology, 196(7), 453-472.

Entry #1143: Wilting our Coffee: the evolution of *Fusarium xylarioides* and its effectors

Title of Project
Wilting our Coffee: the evolution of *Fusarium xylarioides* and its effectors
This project going to be...
Experimental (lab/field)
Full Name of Supervisor
Pietro D SPANU
Institution Department and Address
Exhibition Rd London, London SW72AZ United Kingdom Map It
Telephone
07964007476
Email
p.spanu@imperial.ac.uk
Position held
Professor of Molecular Plant Pathology
Full name of the day to day supervisor and/or arrangements for supervision
Lily Peck
Date of Project Commencement
04/07/2022
Duration (weeks)
8
Brief Description of Project
Coffee has become everyone’s necessity in the morning. It is more than just caffeine that helps us to get through a morning. While we are drinking this bean juice, coffee trees and coffee farmers are fighting against the coffee wilt outbreak. Coffee wilt is caused by a parasitic fungal pathogen called Furasium xylarioides. This fungal pathogen induces death

in the vascular system of the coffee tree so it cannot properly transport water and nutrients to its fruits and leaves. A limited supply of nutrients means fewer fruits (where we get coffee beans) grow, if any at all. This fungus has evolved and adapted to infect coffee species for almost 100 years. This process is called 'Co-evolution', the coffee evolves to avoid being infected as the fungus also evolves to continue infecting the coffee species. This project is trying to understand how this pathogen evolves to become hostile and infectious to coffee by looking at the effector of the pathogen. Effectors are proteins secreted by the plant pathogen to help them infect the plant. The understanding of the effector, as well as co-evolution between this fungus and coffee, potentially leads to improvements in coffee breeding to be more immune to this fungus and potentially any other fungi. Hopefully, this knowledge will help farmers in selecting their coffee varieties and alleviate the result of wilt outbreaks.

The aim of this project is to test both host shifts and pathogenicity using a list of 65 recently described putative effectors as well as infection assays using fungal strains collected over the past 70 years. The first part of this project will involve the selection of target genes and their controls. The second part will involve developing methods for initiating quantitative infection in tomato fruits and measuring time course gene expression in the plant.

Throughout the project, a wide range of techniques will be implemented, which are bioinformatics (designing and testing primer pairs, analysing genomics data), plant disease skills (culturing fungal strains, pathogenicity assays) and molecular biology skills (PCR, qRT-PCR).

Attach the recommended reading for the project

1 Peck, L. D., Nowell, R. W., Flood, J., Ryan, M. R. & Barraclough, T. G. Historical genomics reveals the evolutionary mechanisms behind multiple outbreaks of the host-specific coffee wilt pathogen Fusarium xylarioides. BMC genomics 22, 1-24, doi:https://doi.org/10.1186/s12864-021-07700-4 (2021).

Entry #1141: Resistance is Futile: searching for Fusarium Head Blight susceptibility factors in wheat.

Title of Project

Resistance is Futile: searching for Fusarium Head Blight susceptibility factors in wheat.

This project going to be...

Experimental (lab/field)

Full Name of Supervisor

Paul Nicholson

Institution Department and Address

John Innes Centre, Norwich Research Park
Norwich, Norfolk NR4 7UH
United Kingdom
Map It

Telephone
01603450000
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paul.nicholson@jic.ac.uk
Position held
Group leader
Full name of the day to day supervisor and/or arrangements for supervision
Supervision will be shared between Paul Nicholson and Roshani Badgami (2nd year PhD student). Both PN and RB have office space adjacent to the laboratory. The student will be provided with desk space in the office alongside RB as well as a separate space within the laboratory. The student will have daily contact with both supervisors.
Date of Project Commencement
15/06/2022
Duration (weeks)
10
Brief Description of Project
Over 24 million tonnes of global wheat crops succumb to a destructive fungal disease known as Fusarium Head blight (FHB) annually. The fungal pathogen, Fusarium graminearum, causes premature spike bleaching and produces mycotoxins that accumulate in grain, directly affecting yield and safety. While there have been decades of research into FHB resistance, developing highly-resistant wheat has been challenging. A promising avenue for achieving host resistance is to identify plant genes permitting pathogen success. These genes are known as susceptibility factors. Deletion or mutation of these susceptibility factors can result in recessive resistance that can be both broad-spectrum and durable. This 10-week project aims to refine promising FHB susceptibility loci on the chromosome arms 4DS and 7AS. Hexaploid wheat can withstand large deletions in its sub-genomes. Therefore, the student will screen a selection of gamma-irradiated wheat deletion lines with overlapping deletions spanning these loci (lines already sown). This will involve hands-on experience assessing FHB disease progression in the polytunnel and analysing data using R. The extent of the susceptibility factor-containing deletion will be determined by KASP PCRs. This work will refine susceptibility loci to small regions and allow the identification of individual potential candidate genes. Candidate genes will be further elucidated by gene expression analysis of publicly available RNA-seq data. As little is known about host FHB susceptibility factors, this project will help unravel molecular dialogues during host-pathogen interactions and how Fusarium exploits its wheat host. Owing to collaborations of the Nicholson lab with industry, the results will be of immediate benefit for developing robust future wheat varieties. The project will take place in the Nicholson Lab at the John Innes Centre (JIC), Norwich. The JIC is a centre for cutting-edge research in plant science and microbiology. This project provides a fantastic opportunity to be part of a fun lab in a bustling science community.
Attach the recommended reading for the project

Review on Susceptibility genes:
Van Schie, C., Takken, F. (2014) Suceptibility genes 101: how to be a good host. Annu Rev Phytopathol. 52: 551-81. doi: 10.1146/annurev-phyto-102313-045854.

FHB susceptibility factors:
Fabre F, Rocher F, Alouane T, Langin T, Bonhomme L. (2020) Searching for FHB Resistances in Bread Wheat: Susceptibility at the Crossroad. Front Plant Sci. 11:731. doi: 10.3389/fpls.2020.00731.

Potential FHB susceptibility locus on chromosome arm 4DS:
Hales, B., Steed, A., Giovannelli, V., Burt, C., Lemmens, M., Molnár-Láng, M., Nicholson. P/ (2020) Type II Fusarium head blight susceptibility conferred by a region on wheat chromosome 4D, Journal of Experimental Botany. 71(16):4703-4714 https://doi.org/10.1093/jxb/eraa226

Potential FHB susceptibility locus on chromosome arm 7AS:
Chhabra B, Tiwari V, Gill BS, Dong Y, Rawat N. (2021) Discovery of a susceptibility factor for Fusarium head blight on chromosome 7A of wheat. Theor Appl Genet. 134(7):2273-2289. doi: 10.1007/s00122-021-03825-y. Epub 2021 Apr 8. PMID: 33834252.