WESTERN SYDNEY UNIVERSITY

Hawkesbury Institute for the Environment Summer Scholarship Research Program 2021 Project Lists

Project 53: Foraging behaviour of honeybees and native bees on a communal floral resource

Supervisor(s): Amy-Marie Gilpin - a.gilpin@westernsydney.edu.au
Principal Supervisor

Kate Umbers - k.umbers@westernsydney.edu.au
Second Supervisor

Project Description

Native pollinators are recognised as providing a huge economic benefit to Australian agriculture. However, currently, the introduced European Honeybee (Apis mellifera) is frequently brought in to cropping areas to provide pollination services when the crop is in bloom. It is currently unknown how this sudden influx of introduced pollinators can affect the foraging behaviour of native pollinating bees when they are co-foraging on a communal floral resource such as apple flowers. We aim to determine whether there is competitive displacement of native insects at a flower level.

This project will involve analysing footage of native and introduced bees in apple orchards to determine their foraging behaviour. The project will provide a unique opportunity to integrate pre-recorded field-collected data with computational image analysis and allows students to learn classic behavioural survey methods and analysis.

Project Aims

This project aims to determine how native and introduced bees interact whilst co-foraging on a communal floral resource and determine whether there is competitive displacement of native pollinating bees.

Project Methods

This project will take advantage of pre-recorded footage of insects visiting apple flowers and will provide a unique opportunity to integrate field-collected data with computational analysis.

Opportunity for Skill Development

This project will provide the student with a sound understanding of the fundamentals of research – from hypothesis development to experimental design and data collection – providing a solid framework for future participation in HDR study in the area of behaviour and pollination ecology. The student joining this project will have opportunity to learn how to collect data using image processing, analyse experimental data and communicate their findings.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

The essential criteria for student participation in the Summer Scholarship Program are sufficient for skill pre-requisites. Bachelor of Science, or Bachelor of Natural Science students are most likely to benefit from this project, however students with an expressed interest in insects, plants, ecology, and/or behaviour research who are enrolled in other programs are welcome to apply. Students should be willing and able to work from home on a computer able to handle image processing software (all free), with access to zoom video for meetings with supervisors.

Project 54: The effect of fire history on the flowering phenology of Eucalypts

Supervisor(s): Amy-Marie Gilpin - a.gilpin@westernsydney.edu.au
Principal Supervisor

Rachael Nolan - Rachael.nolan@westernsydney.edu.au
Second Supervisor

Project Description

Eucalypts are vital for numerous vertebrates and invertebrates who rely upon the prolific floral resources that they produce. However, to date, very little research has investigated the impact of fire on the flowering phenology and production of these floral resources. The 2019-2020 bushfires affected over 5 million hectares of NSW National Parks, state forests and private property much of which is dominated by Eucalypt forest. This represents a significant loss of floral resources for a wide range of animals over a large geographical area. Therefore, this project aims to quantify the flowering phenology of Eucalypts with different fire histories including time since fire and intensity in order to determine the impact of fire on floral resource production. This project will use photographic data collected from the field along with computational image analysis which will allow the student to learn remote survey methodologies.

Project Aims

This project aims to determine the effect of fire history on the flowering phenology and therefore floral resources of Eucalypts.

Project Methods

This project will use pre-existing photographic data collected in the field and will utilise computational image analysis to identify the effect of fire history on the flowering phenology including timing of bud development and duration of flowering of key Eucalypts. This project will allow students to develop remote data analysis skills as well as learn key plant and pollinator survey methods.

Opportunity for Skill Development

This project will provide the student with an understanding of the fundamentals of research – from hypothesis development to experimental design and data collection – providing a solid framework for future participation in HDR study in the area of plant- pollination ecology. The student joining this project will have opportunity to learn how to collect data using image processing, analyse experimental data and communicate their findings in a variety of ways.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

The essential criteria for student participation in the Summer Scholarship Program are sufficient for skill pre-requisites. Bachelor of Science, or Bachelor of Natural Science students are most likely to benefit from this project, however students with an expressed interest in plants, ecology, and/or computer based analysis research who are enrolled in other programs are welcome to apply. Students should be willing and able to work from home on a computer able to handle image processing software (all free), with access to zoom video for meetings with supervisors.

Project 55: Can we use leaf traits to predict fuel moisture content across woodland communities in SE Australia?

Supervisor(s): Anne Griebel - a.griebel@westernsydney.edu.au
Principal Supervisor

Rachael Nolan - Rachael.nolan@westernsydney.edu.au
Second Supervisor

Project Description

The Australian "Black Summer" fire season of 2019–20 burned an estimated 7.2 Mha of fires in the temperate south-east of the country. The fires coincided with the driest and hottest year on record for Australia and were precipitated by record-breaking fuel dryness and fire weather. Quantifying when and where forests are approaching critical dryness levels is thus vitally important for assessing the risk of bushfires and for planning management burns. Despite the importance of fuel moisture in driving fire activity, it remains challenging to forecast live fuel moisture content (LFMC, the moisture content of live foliage). Current approaches to modelling LFMC as a function of soil dryness have mixed success because they do not account for the differing responses of plants to declining soil moisture. Surprisingly, there has been little research on the Eco physiological drivers of LFMC.

Recent studies in Mediterranean tree and shrub species (in north-east Spain) found that LFMC could be modelled from plant physiological traits, and using a potted plant experiment it was demonstrated that foliar water potential can be used to model LFMC in eucalypt species. This experiment found that an important factor affecting the relationship between species were leaf traits such as leaf mass per area, turgor loss point, etc., which is an exciting step forward in developing a physically-based LFMC model for Australia's forests and woodlands.

With this Summer Scholarship, we propose for a student to join our fuel moisture modelling team in testing whether leaf traits can help to constrain the variation in LFMC across a range of forest communities within the Blue mountains.

The proposed project is to:

- Investigate the relationship between maximum LFMC (analogous to saturated water content) and leaf traits (such as leaf mass per area, leaf dry matter content and leaf density) across a large range of species (minimum of 10). Samples will be collected within mixed woodland communities along a climate gradient across the Blue mountains (pending covid restrictions). Field sites will be aligned with current research projects from HIE's Bushfire group. If government restrictions limit movement, then local species can be accessed that are growing at Hawkesbury campus facilities
- For a subset of species, measure the relationship between LFMC and leaf water potential on cut, rehydrated leaves as they dry on a bench top ("pressure-volume" curves). This will be on a maximum on six species.
- Establish statistical relationships between LFMC, climate variables (available from the Bureau of Meteorology) and leaf traits that were derived from this project.

It is anticipated that this study will result in a peer-reviewed publication (target is International Journal of Wildland Fire), which the student will be a named co-author on. Additionally, this research will provide an important "proof-of-concept" which will underpin further field-based studies of the drivers of LFMC. These field-based studies will then directly inform the development of a predictive model of LFMC for south-east Australian forests. These research activities are part of the "Forecasting Live Fuel Moisture content, the on/off switch of forest fires" research project led by Dr Anne Griebel, A/Prof Matthias Boer and Dr Rachael Nolan.

Project Aims

The specific aims of the research project are:

i. To establish relationships between observed live fuel moisture content (LFMC) and leaf traits
ii. Model the relationship between LFMC and leaf water potentials
iii. Examine how observed LFMC and leaf water potentials can be predicted using climate variables (available from the Bureau of Meteorology) and leaf traits that were derived from this project.
iv. Provide an opportunity for a student to be involved in all aspects of research from establishing an experiment through to analysing and interpreting results.

Project Methods

The student will be directly involved in all aspects of this study.

- Collecting foliage for saturated water content and leaf trait measurements, rehydrating foliage, and undertaking measurements
- Undertaking pressure-volume curves
- The student will be responsible for managing the data collected during the experiment. The student will analyse the data using the software package R.

Opportunity for Skill Development

The student will learn the following skills:

- Handling and analysing of field samples from native forest and woodlands
- Standard lab techniques that are frequently applied in fire research and in the field of ecophysiology
- Data analysis using the R-language and associated software packages
- Data management
- Scientific writing

Students are required to have the following skills/meet the following pre-requisite(s) to apply

All necessary skills can be acquired during the project. However, preference will be given to candidates that have at least two of the following attributes:

- A keen interest to learn about Australian native vegetation and in working outdoors
- Experience with field-based research (e.g. through active participation in practical units)
- Experience with laboratory techniques, esp. relating to plants
- Experience in programming (using R, python, matlab etc.)
- A valid driver license

Project 56: How does growth and turnover of branches affect forest ecosystem responses to elevated CO2?

Supervisor(s):

Ben Smith - ben.smith@westernsydney.edu.au
Principal Supervisor

Belinda Medlyn - b.medlyn@westernsydney.edu.au
Second Supervisor

Rachael Nolan - rachael.nolan@westernsydney.edu.au
Third Supervisor

Project Description

How does growth and turnover of branches affect forest ecosystem responses to elevated CO2?

The student project will be integrated with the PhD project of Ms Klaske van Wijngaarden, a cotutelle student jointly enrolled at WSU/HIE and the University of Birmingham, UK, supervised by Professors Smith and Medlyn. The project aims to understand how changed tree growth patterns and productivity in response to higher atmospheric carbon dioxide concentrations impact the production of bark, branches and woody material in contrasting forest ecosystems of the UK and Australia. The student funded through this scholarship will sample production of fine and coarse woody litter from control and elevated CO2 (eCO2) treatments in the EucFACE eCO2 ecosystem experiment. The resulting data will enable a comparison with corresponding data from the BiFOR eCO2 experiment established in an oak forest in the UK, collected by Ms Wijngaarden, who is unable to enter Australia to perform the field work herself at present owing to covid-related border restrictions. This student project will focus on turn-over of the EucFACE species, in particular bark, branches and fine woody material and will further investigate the size distribution on the forest floor of the different treatment rings at the EucFACE research site. The project will provide the student with exposure to an exciting and globally unique experimental field facility in iconic Cumberland Plains woodland, representative of the Western Sydney environment, and the opportunity to gain experience in ecological field techniques and experimental design. Plotting and tabulation of the data for quality control and the preliminary identification of experimental treatment effects will form part of the student work, under the guidance of the supervisory team.

Project Aims

The goal of this project is to determine if there is any difference between the production and turn-over of woody material over time under ambient and elevated CO2 concentrations and to provide a dataset enabling comparisons between forests in Australia and the UK. The baseline inventory will help the cotutelle PhD student to whose thesis work the student project is linked to acquire an initial time series from EucFACE and will help the student gain experience in setting up a fieldwork experiment and have opportunity to work with data gathered at EucFACE and at Birmingham Institute of Forest Research (BIFoR) FACE. Although the covid pandemic and subsequent travel restrictions have been a challenge to this intercontinentally spanning project, it's still very much the aim to connect and learn as much as possible from the two forest eCO2 experiments, which are the only facilities of their kind anywhere in the world.

Project Methods

The student will perform a line-transect inventory, recording diameters, length and notes of species and decay category of coarse woody debris (CWD) litter within control and eCO2 treatment plots (rings) of the EucFACE experiment. The transect will be replicated in each of the 6 rings at EucFACE and inventory of recent fallen material will be made at intervals of about two weeks through the summer period. If the schedule of the student, logistics and time permits, canopy mapping or processing of data derived from earlier made terrestrial-laser scanning (TLS) will complement the litter inventory work. In case the student is interested in gaining more lab-work experience the analysis of moisture content in twigs, bark and/or bigger woody samples can be arranged, providing additional useful data to understand tree responses.

Opportunity for Skill Development

The student will gain experience in planning, establishing and executing a field sampling strategy as part of a large-scale ecological field experiment, as well as related research skills in the lab, point data processing and time series analysis, subject to time and interest by the student. Combining and comparing the data gathered from EucFACE and BIFoR eCO2 experiments is the foundation of this student project and the PhD project it contributes to. Guidance will be provided by the local supervisor team, who are leading experts in ecosystem function and elevated carbon dioxide studies, and regular interactions with Ms Van Wijngaarden will be arranged for additional mentorship and collaborations.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

Training in all relevant field, lab and data analysis will be provided. No pre-existing specialised skills are required. The student should be willing to work outdoors and have a general interest in ecology and field-based experimental science.

Project 57: Heat stress of urban plantings: finding sustainable planting types

Supervisor(s): David Ellsworth - D.Ellsworth@westernsydney.edu.au
Principal Supervisor

Tim Carroll
Second Supervisor, External Partner, **Andreasens Green Wholesale Nurseries**

Project Description

With a succession of recent hot summers in Sydney and the spectre of climate change, a key challenge for greening Australia's urban and peri-urban areas is to ensure that future plantings with trees are successful. This requires ensuring that our tree plantings can tolerate hot and dry climate conditions that can be expected in the future. This summer project is an extension of the Which Plant Where project (http://www.whichplantwhere.com.au/) involving 6 scientists here at Western Sydney and involving partners Macquarie University, City of Penrith, and NSW government, and involves a partnership with a major Sydney-based plant nursery, Andreasens Green. The Which Plant Where project aims to inform the community and local councils about selecting the right plants for the right place, with an eye on the future for more sustainable urban areas. We are seeking to find out under what conditions current favourite plant varieties are unlikely to thrive under the more extreme climates that Australian cities face, and stress-test major landscape species to find ideotypes for new species and varieties to be planted for urban greening.

The main scope of work for this summer project involves work in the local tree nursery Andreasens Green, and on trees from Andreasens that are tagged and planted out in local council developments, Western Sydney's Hawkesbury campus, and/or alongside road verges. We will quantify physiological/health indicators for normal growing conditions and during hot summer periods for 15-25 species of major horticultural trees. The student will get training with scientific equipment and stress-test major landscaping tree species to test how plantings handle adverse conditions (heat and dry) and understand what plantings could be expected to fail and whether improving nutrition can improve survival under stress. The work will involve measurements with high-tech equipment (chlorophyll fluorescence and greenness, IR leaf temperature, and soil moisture and water-use) made on nursery stock and out-planted trees in conjunction with environmental measurements like local temperature and moisture conditions. The project is aligned with a research partnership agreement involving postgrad students with Everris Australia, and ARC-Linkage proposal (pending) as well as with the WPW project. The student will receive some backing from the relevant grants and interact with the industry partner and some of the science team at HIE.

Project Aims

Tree stock can be influenced by watering, nutrition, climate, as well as other factors related to how they are cultivated. Our aims are:

- Gain experience with scientific equipment
- Measure some key indicators of health/vigour for nursery stock from across different pot sizes and from pot to the field/urban environments.
- Determine the norms of biological variability for well-watered, unstressed nursery trees
- Learn how to summaries and report your findings to others

Project Methods

This project uses plant Eco physiological techniques as the basis for the measurements.

The measurements involve leaf-level measurements in a local nursery, and in the field (on campus and in nearby development areas). Leaf greenness (SPAD meter), leaf chlorophyll fluorescence with a pulse-amplitude modulated fluorometer (MINI-PAM-II/B, Heinz Walz GmbH, Effeltrich, Germany) will be used for the measurements, Leaf temperature will be made using a point-measurement infrared thermometer, including during high-heat conditions as they may occur during the summer period. The student will be contributing to the larger bulk of data collection to be undertaken by a team of researchers at HIE, including the project supervisors.

Opportunity for Skill Development

This project will introduce the student to the fields of plant physiology and urban ecology. The student will develop skills using several pieces of scientific equipment, as well as skills for proper data collection, organisation, hypothesis-testing and analysis.

- The student gains the ability to make and interpret scientific measurements
- The student will see how inclement environmental conditions, and also
- The student learns about the precision and accuracy of measurements. The student gets the opportunity to work with a few different electronic sensors
- The student learns how to analyse the data quantitatively, and present it appropriately

The student will learn to function independently after receiving proper guidance, learn how to work in a team, and learn how to contribute to a research team effort.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

Student candidates should be willing to work in the field while following safe-work practices. Work will sometimes be involving brief inclement conditions such as hot days during summer. Ability to drive (holder of an NSW or other Australian state's drivers' licence).

Project 58: Improving soil water for enhancing plant growth under water stress

Supervisor(s): David Ellsworth - D.Ellsworth@westernsydney.edu.au
Principal Supervisor

Will Pearce
Second Supervisor, External Partner, **Everris Australia Pty Ltd (ICL-Group Australasia)**

Renee Prokopavicius - r.prokopavicius@westernsydney.edu.au
Third Supervisor

Project Description

New plantings in Western Sydney and other urban areas across Australia face periods of water shortages that

are growing more frequent, longer lasting and more dangerous to plant survival. We ask, can soil adjuvants assist with plant survival and growth for horticultural plantings in an effort to enhance urban greening? The main scope of work for this summer project involves an experiment on 3 popular horticultural shrubs to test watering × soil moisture adjuvant interactions. Major outcomes will involve testing whether wetting agents can improve growth and water-use efficiency during drought, which enables industry recommendations regarding water augmentation agents to be implemented in horticulture and urban greening and renewal.

The student will quantify stomatal conductance, plant water use, soil moisture and growth to understand whether the augmentation of soils with wetting agents or wetting agents improves plant performance. The work will proceed using advanced (45 L) shrubs obtained from a local horticultural nursery, for 2 popular shrub species (Callistemon, and Lagerstroemia). The student will get training with scientific equipment and stress-test major landscaping tree species to test how plantings handle adverse conditions (drought) and the effects of the soil moisture adjuvants. The work will involve measurements with high-tech equipment (leaf porometer/water-use, leaf pressure chamber, and soil moisture) made on nursery stock subjected to drought × soil water adjuvant treatments in conjunction with environmental measurements like local temperature and moisture conditions. The research is aligned with a growing research partnership between Western Sydney University and Everris Australia/ICL-Group via studentships and an ARC Linkage grant. The student will interact with the industry partner and some of the science team involved in horticultural research in HIE.

Project Aims

Tree stock can be influenced by watering, drought and climate, as well as other factors related to how they are cultivated. Our aims are:

- Gain experience with scientific equipment
- Measure some key indicators of water status for common nursery plant stock during summer conditions.
- Determine the effects of soil water additives on nursery shrubs
- Learn how to summarise data and report your findings to others

Project Methods

This project uses plant Eco physiological techniques as the basis for the measurements. The measurements involve leaf-level measurements of stomatal conductance and transpiration water-use for shrubs from a local nursery in the field on campus at the Hawkesbury campus. Shrubs will be acquired from a local nursery and subject to a structured set of experimental treatments: well-watered (WW), droughted (D), WW + planted with water crystals, D + planted with water crystals, WW + wetting agent, D + wetting agent. This design lends itself well to robust statistical tests. All plants will be subject to either a natural heatwave outdoors (> 40 C), or an experimental heatwave in the glasshouse, to test how well they perform under these conditions.

Frequent watering and monitoring of water status of the shrubs is required to maintain experimental drought. Leaf water potential will also be monitored during summer conditions. Growth will be determined by allometric measurements (diameter, twig growth, etc.). The student will be contributing to the larger bulk of data collection to be undertaken by a team of researchers at HIE, including the project supervisors.

Opportunity for Skill Development

This project will introduce the student to the fields of plant physiology and urban ecology. The student will develop skills using several pieces of scientific equipment, as well as skills for proper data collection, organisation, hypothesis-testing and analysis.

- The student gains the ability to make and interpret scientific measurements
- The student will see how inclement environmental conditions, and also
- The student learns about the precision and accuracy of measurements. The student gets the opportunity to work with a few different electronic sensors
- The student learns how to analyse the data quantitatively, and present it appropriately

The student will learn to function independently after receiving proper guidance, gain exposure to relevant industry professionals, and learn how to contribute to a research team effort.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

Student candidates should be willing to work in the field while following safe-work practices. Work will sometimes be involving brief inclement conditions such as hot days during summer.

Project 59: A genetic biobank for threatened waxcaps (Hygrocybe)

Supervisor(s): Jeff Powell - jeff.powell@westernsydney.edu.au
Principal Supervisor

Jordan Bailey
Second Supervisor, External Partner, **NSW Mycology Herbarium (DPI, Orange) & NSW DPIE Biodiversity and Conservation Division**

Project Description

The NSW Mycology Herbarium and the Biodiversity and Conservation Division of Department of Industry, Planning and Environment are collaborating with Western Sydney University to develop a genetic biobank of threatened Hygrocybe within Greater Sydney. There are nine species of threatened fungi and a Critically Endangered Hygrocybeae Community of Lane Cove Bushland Park, from the family Hygrophoraceae found within the Sydney Basin IBRA Region. The nine listed fungi include: Hygrocybe collucera, Hygrocybe griseoramosa, Hygrocybe lanecovensis, Camarophyllopsis kearneyi (endangered) and Hygrocybe aurantipes, Hygrocybe austropratensis, Hygrocybe reesiae, Hygrocybe rubronivea, Hygrocybe anomala var. ianthinomarginata (vulnerable). The Threatened Ecological Community is comprised of more than 30 species of fungi in the family Hygrophoraceae. More information on the TEC can be found here. This project is funded under the NSW Government Saving our Species Program. Further information on this program the Hygrocybe project can be found here.

The conservation project aims to maintain Hygrocybe diversity, extent and habitat (vegetation and soils) at priority sites. To achieve this, we require a basic understanding of the existing Hygrocybe diversity and distribution at each site. As such the NSW Government has partnered with the NSW Mycology Herbarium and Western Sydney University to pair morphological identification of samples with DNA sequencing and develop a reference collection and DNA Biobank.

Project Aims

- Add further specimens to a growing reference collection of Hygrocybe species collected from Greater Sydney and the Blue Mountains
- Complement this collection with DNA sequences obtained from those specimens, which will then be used for assessment of future specimens and other types of environmental samples
- Use this information to provide management recommendations at priority sites under the Saving Our Species program, with the aim to maintain Hygrocybe diversity and extent

Project Methods

The student will visit the Herbarium in Orange to inspect these collections, comparing samples to established keys. They will use commercial DNA extraction kits to purify DNA from selected samples and will then perform polymerase chain reactions to amplify target DNA from these sample, being taught this process by Jordan and other members of her group. They will perform Sanger sequencing on extracts using sequencing primers associated with established DNA barcodes (internal transcribed spacers and the 28S large subunit of the ribosomal RNA gene), being taught this process by Jeff and other members of his group. They will then be taught how to analyse DNA sequences using software that is licensed by DPI and WSU) by Jordan, Jeff and members of their groups.

Opportunity for Skill Development

The student will have the opportunity to learn state-of-the-art methods currently used in relation to biodiversity conservation and management from Jordan, who is actively using these methods in multiple projects relating to conservation and biosecurity. The student will also have the opportunity to participate in subsequent field surveys with Meagan; these surveys typically occur between April and August, which is when most fruiting occurs.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

The student is expected to be enrolled in a degree in either the School of Science or the School of Medicine. Students entering their final year of study will receive preference.

Project 60: The very hungry fly: the nutritional requirements of pollinating blow flies

Supervisor(s): Jon Finch - j.finch@westernsydney.edu.au
Principal Supervisor

James Cook - James.cook@westernsydney.edu.au
Second Supervisor

Project Description

Flies (Diptera) are increasingly recognised as important pollinators. Flies can deposit large amounts of pollen and have several distinct advantages over honeybees in protected cropping systems, like glasshouses. These advantages include being safer to work with and being easier to ship and deploy. Flies can also be easily reared on a variety of inexpensive waste products, such as manure. However, major questions remain about fly pollination. For example, what resources are flies visiting flowers for and how does this change throughout their lives and between sexes? Foraging behaviours like these have been studied for decades in honeybees but very rarely in flies. We aim to conduct a simple experiment to quantify how nectar and pollen consumption differs as flies age and between sexes.

We hypothesise that young flies probably have higher energetic and nutritional demands compared to older flies because of their rapidly developing reproductive tissues (eggs and sperm). As such, young flies may have higher nectar and pollen consumption rates than older flies. Conducting this experiment will help us to understand how flies use floral resources, with implications for how we manage fly pollinators in protected cropping. If younger flies do have greater nectar requirements compared to older flies, then new young flies might be added more frequently in protected cropping environments to promote foraging behaviour and pollination.

Differences in resource use may also occur between male and female flies. This is because in many species maternal investment in reproduction is often greater than in males. As such, we hypothesise that female flies may have greater resource requirements than male flies. If this is the case, it suggests it may be beneficial to establish all female lines of flies for use as pollinators in protected cropping using Wolbachia bacteria or other biotechnologies.

The proposed research will help to inform our research at HIE as part of the "flies as managed pollinators project", a major collaboration between Hort Innovation Australia, the University of New England, the Department of Primary Industries and Regional Development in Western Australia, and Seed Purity in Tasmania.

Project Aims

- To characterise the nutritional requirements of adult blow flies and determine the effects of age and sex on resource use.
- To provide our findings to growers and other industry partners to inform their use of fly pollinators
- To publish our research in a peer reviewed journal

Project Methods

Flies (Calliphora stygia) will be obtained as pupae from a supplier in South Australia. Following emergence, adult flies will be stored in plastic takeaway containers under laboratory conditions (n=30). Artificial nectar (caster sugar: tap water solution) and pollen will be placed into plastic syringes. The open ends of the syringes will be inserted into holes cut into the takeaway containers for the flies to feed on for the duration of the experiment.

The syringes will be removed and weighed every 2-3 days over the duration of the lifespan of the adult flies (~30 days). By recording the changing weight of the syringes, we will be able to quantify the rate of consumption of pollen and nectar as flies age. Controls (plastic containers with pollen and nectar syringes but without flies) will be used to account for evaporation or other processes that might alter the weight of the syringes. After 30 days the flies will be humanely euthanized by freezing, after which their sex will be determined by microscopy to see if this also plays a role in nutritional demand.

Data will be analysed in the statistical software package R using ANOVA tests.

Opportunity for Skill Development

In conducting the experiment, the student will learn how to rear, handle, identify and work with live insects.

In addition, the student will work closely with professional researchers to gain invaluable experience in conducting controlled experiments under laboratory conditions in order to test clear scientific hypotheses. The student will also be instructed on how to collect, format and analyse the resulting data. The nature of this simple experiment is extremely novel and is likely to result in a peer reviewed publication. If so, the student will be invited to participate in the writing of the manuscript and will be included as an author. This experience will be invaluable to their future career in science.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

No specific experience is required but a general interest in entomology and/ or protected cropping is preferable.

Project 61: Are urban forests equally distributed in Sydney? A climatic and socio-demographic approach.

Supervisor(s): Manuel Esperon-Rodriguez - m.esperon-rodriguez@westernsydney.edu.au
Principal Supervisor

Sally Power - s.power@westernsydney.edu.au
Second Supervisor

Project Description

Urban forests include trees planted in street, garden and park contexts across our cities. These are essential elements of liveable, sustainable cities as they provide multiple ecosystem services, such as mitigation of urban warming, carbon sequestration and improved air quality, as well as having positive impacts on human wellbeing. We have observed that at street level, tree shade can reduce up to 3.7 °C of air temperature and 20 to 35 °C of surface temperature compared to non-leafy streets. Not all suburbs, however, have a similar degree of tree canopy cover. Within Greater Sydney, canopy cover varies from 10-40% among suburbs. This variation may be related to socio-economic factors, including wealth, educational qualifications, housing age and home ownership levels.

Suburbs within Greater Sydney are rich in diversity, culture and ethnicity, and the perception of urban forests and green space in different suburbs can also be related to cultural and social background, which may affect the stewardship of urban forests.

Additionally, Greater Sydney has a climatic gradient from the wet and cool coast to the dry and hot inland. Given that there are thermal and aridity limits to tree species' distributions, understanding the effects of climate on urban forestry is critical. There are reasons to assume that those climatic differences also influence the species composition of urban forests.

Therefore, disentangling the relationships between the distribution of urban tree species and climatic and socio-economic factors is critical for informing urban planning and for moving towards more sustainable and equitable cities.

Project Aims

This project aims to answer three questions:

1. Do tree abundance and species composition vary among suburbs in Sydney?
2. How do socio-economic characteristics affect tree abundance and species diversity among suburbs?
3. Do patterns in tree abundance and species diversity reflect differences in climate- across Sydney's suburbs?

Project Methods

Previously, our team collected data on urban tree inventories across Greater Sydney. We will be using these tree inventory data to obtain species and composition information of urban forests across multiple suburbs.

We will use existing climatic data (i.e. temperature, humidity and precipitation) for respective suburbs, to evaluate relationships between climatic conditions and urban forest metrics.

Socio-demographic data will be accessed from the Australian Bureau of Statistics, to evaluate relationships with urban tree canopy cover.

This will be a desk-based project. The student will be involved in collecting and handling these large data sets. The project will involve data cleaning, formatting inventory data from different suburbs and analysis to answer our three main questions.

The student will also have the opportunity to develop additional research questions based on their interests and the data we will provide.

Opportunity for Skill Development

The student will develop a broader understanding of urban greening and will learn how to handle and process large data sets and develop data management skills. Along with these, the student will develop skills in basic R- software, scientific report writing and importantly will have experience of working within a large, industry-funded project. In addition, they will have the opportunity to learn about a wide range of ongoing projects run by affiliated Ph.D. students and post-docs conducting research on related questions, gaining experience of how to work as part of a wider research team. Students will be supported in connecting ecological theory to practice, developing research questions, hypotheses and predictions and, if interested, learning about writing for a general audience and the scientific community.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

The student must be willing to work with large datasets. Experience in working with different software e.g. excel and R will be highly appreciated.

Project 62: Now that's a cool trait! Evaluating how tree canopy characteristics contribute to urban heat mitigation

Supervisor(s): Manuel Esperon-Rodriguez - m.esperon-rodriguez@westernsydney.edu.au
Principal Supervisor

Sally Power - s.power@westernsydney.edu.au
Second Supervisor

Project Description

More than 4.2 billion people live in urban areas, representing ~3 percent of the Earth's land area. As the human population grows, cities around the globe will continue to expand their footprints and increase their demands for goods and services. Ecosystem services, such as temperature regulation, are provided by urban forests in cities worldwide. However, the combined effects of the urban heat island (UHI) effect and climate change are driving record-breaking, extreme temperatures in urban areas. Such conditions pose considerable health risks for city dwellers. To mitigate urban heat, increasing tree cover is a practical strategy. Tree canopies reflect and transmit solar radiation and, by providing shade, minimise the amount of solar radiation absorbed by sub-canopy surfaces resulting in cooler conditions compared to areas experiencing full sunlight. Research carried out by a PhD student in the Hawkesbury Institute for the Environment has shown that trees with dense, wide canopies are better at reducing air and surface temperatures, yet much remains to be explored about which species of tree, with which associated canopy and leaf traits, can provide the greatest cooling benefits in our increasingly hot, Western Sydney landscape. The Which Plant Where (WPW) project, funded by Hort Innovation Australia, aims to increase the knowledge base that will help make our cities more liveable not only now but under future climatic conditions. An essential part of having a liveable city is the maintenance (and expansion) of green spaces that provide many core benefits to society.

This summer scholarship project focuses specifically on how different plant attributes are related to the provision of ecosystem services on a local scale, focusing specifically on how different leaf and canopy-level traits are related to air temperature reduction on a local scale. The student joining our project will examine trees' cooling benefits, in a standardised, common-garden experiment set up at the Hawkesbury Campus - with student interest driving additional or alternative data collection. A variety of measurement techniques (e.g. tree biometrics and morpho-anatomical and physiological traits) will be used to obtain data on which vegetation traits (e.g. leaf area index, height, canopy density) are associated with the maximum provision of cooling benefits. The student will be introduced to the concepts of ecosystem services and will have the opportunity to learn about survey design, sampling strategies, data management and develop their skills in the relevant statistical techniques and software. This project also offers enormous opportunity benefits to the student via interactions with a team of post-docs and Ph.D. students contributing to related questions as a part of the wider WPW project.

Project Aims

To understand which tree morphological and physiological characteristics are associated with maximum cooling benefits.

Project Methods

This project involves fieldwork, sampling, and data collection from the Hawkesbury Urban Forest research facility at Western Sydney University's Hawkesbury Campus. All fieldwork will be supervised by the primary supervisor. Research conducted for this project is mostly undertaken outdoors, so a willingness to spend time in the field is important for this project.

Data collection for this project will include spot measurements of different biological and physical parameters (such as tree height, diameter at breast height) and sample collection, followed by measuring morpho-anatomical plant traits. This will contribute to a comprehensive evaluation of relationships between species characteristics and local cooling benefits. All these findings will be shared with industry partners and plant nurseries and will help to highlight the importance of having appropriate tree species in our cities to maximise the benefits they bring to urban populations.

Opportunity for Skill Development

This project will provide a sound understanding of the fundamentals of research – from hypothesis development to experimental design and data collection – providing a solid framework for future participation in HDR study in the area of urban ecology. The student joining this project will have the opportunity to tailor their research skills development from measurements to data analysis and inference, focussing on applied topics, such as plant physiology. In addition, they will have the opportunity to learn about a wide range of ongoing projects run by affiliated Ph.D. students and post-docs conducting research on related questions using the WPW framework, gaining experience of how to work as part of a wider research team. As part of the WPW team, they will be supported in connecting ecological theory to practice, developing research questions, hypotheses and predictions and, if interested, learning about writing for a general audience and the scientific community.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

No specific prerequisites.

Project 63: Phenotyping Setaria viridis Hexokinases in Setaria and rice to determine the difference between C3 and C4 sugar sensors

Supervisor(s): Oula Ghannoum - o.ghannoum@westernsydney.edu.au
Principal Supervisor

Lily Chen - L.Chen6@westernsydney.edu.au
Second Supervisor

Project Description

The main purpose of photosynthesis is production of sugars for use in plant growth, maintenance and propagation. During light periods, photosynthetic carbon fixation occurs in the leaf, where these sugars ultimately are transported throughout the plant to be used for cell maintenance, growth and storage. While at night the plant consumes the stored sugars. This indicates that the high level of fluctuation in sugar levels of plant source tissue is partly due to changes in light resources. CO2 concentrations also affects the levels of photosynthetic sugars produced during the day, with higher levels of CO2 available for photosynthesis resulting in a higher accumulation of sugars. In response to high levels of sugars, key photosynthetic genes have been shown to be down regulated. For example, RbcS is down regulated under high CO2 levels, where sugar have accumulated. The steps between sugar levels and transcript regulation are not yet well understood, due to a complex regulatory network of sugar metabolism and sensing genes encompassing many mechanisms other than regulation on photosynthesis. Three known sugar sensors (TOR, HXK and SnRK1) have been proposed to play a role in photosynthetic regulation. Manipulation of these genes in planta, would allow for a mechanism to understand this process.

The objective of this project will be to phenotype plants which overexpress some of the sugar sensing genes in Setaria viridis and Rice. This phenotyping study seeks to determine the effects of these genetic manipulations plant by measuring a range of parameters such as carbohydrate content, transcript abundance, protein abundance, gene expression as well as photosynthetic capacity.

Project Aims

Determine if there are phenotypic difference between the wild type and the plants overexpressing hexokinase

Project Methods

During phenotyping of the overexpression plant lines the plants will be grown for 3 – 4 weeks and plant samples will be harvested for various types of testing. We can determine if there are different levels of transcripts by using RT-qPCR and primer design where the student can perform these processes. Carbohydrates will also be extracted from the plant tissue to be quantified which the student will also participate in. Moreover, the student will help in protein extraction and quantification using SDS-PAGE and Western blotting. In addition, the student will have an opportunity to learn molecular skills and try their hands at genetic transformation of plants alongside Dr Chen.

Opportunity for Skill Development

The student will have an opportunity to measure the expression of different genes within these genetically manipulated plants using RT-qPCR. They will learn how primers are designed and then used to quantify transcript levels. Moreover, the student will have an opportunity to extract sugars from plant material in order to quantify how much of each carbohydrate is present in various plant tissues. The student will also have the opportunity to extract proteins from plants and visualise them using SDS-PAGE and Western blotting techniques to determine if there is a difference between protein abundance between WT and overexpression lines. During these experiments the student will learn to keep a tidy workspace in a laboratory environment and how to keep track of samples to avoid mistakes. Additionally, the student will most importantly be shown how to properly use laboratory equipment and tools such as using the pipette to accurately aliquot liquids. We will also develop the notetaking skills of the student in a proper lab environment to ensure there is no confusion during different processes and samples. The student will also be shown how to minimise contamination of samples to avoid misrepresentation of results. Following experimentation, the student will also be shown how to analyse data correctly, interpret results and how to represent them in a logical manner.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

Basic undergraduate training in biological sciences is sufficient and it is highly desirable if the student has molecular biology skills such as familiarisation with a pipette and an understanding of scientific terms.

Project 64: Linking physiological function of plants with leaf anatomy and aquaporin function

Supervisor(s): Oula Ghannoum - O.Ghannoum@westernsydney.edu.au
Principal Supervisor

Yazen Al-Salman - Y.Al-Salman@westernsydney.edu.au
Second Supervisor

Project Description

The project would build on and complete a larger project started in 2019 to assess genetic controls on water use efficiency in the key C4 crop Sorghum bicolor, as well as elucidate the role of the important membrane proteins called Aquaporins. The project is based on a glasshouse experiment conducted between Oct 2019 and Feb 2020 where 90 Sorghum genotypes were grown, some under drought conditions, and several measurements and samples collected.

The first two parts of the analysis involve analysing gas exchange, biomass and genotype responses as well as the genetic background and signals that coordinate those responses. Those parts are currently being conducted by the secondary supervisor (Yazen) and other collaborators in other institutions.

The student comes in to conduct key remaining research that will complete the analysis of this experiment. Depending on the student's interest and availability, the two remining pieces of research are:

1. Anatomical Work. This concerns the analysis of leaf anatomical features. We have leaf samples currently fixed in ethanol. The student would conduct lab work to obtain leaf surface impression for stomatal anatomy, clear and stain leaves for vein anatomy, and cut and fix leaf sections in wax or resin to obtain leaf cross-sectional anatomy and path lengths.
2. Analysis of RNA expression. The project is based upon diversity in aquaporin alleles. Hence, a key part is to pin point which aquaporin genes are expressed and under which watering condition. The student would perform Polymerase chain reactions and western blots and gel electrophoresis all from snap frozen leaf samples to determine which proteins were expressed and at what levels.

The student would be able to choose either of the above projects, or if time permitting and there is interest, both. We anticipate that performing one of the above would take the entirety of the student placement, but depending on how much of the work the secondary supervisor will conduct alongside the student it is possible that the student can be involved in both sections.

Project Aims

1. Provide the data that will enable the researchers to link genetic background with gene expression of a key membrane protein in an important crop.
2. Enable the possibility of discovering novel genes that regulate key leaf processes in a crop.
3. Elucidate the functional impact of the aquaporin protein family on leaf processes.
4. Properly enable the separation of anatomical and aquaporin influences on leaf conductances and fluxes.

Project Methods

1. Leaf anatomical measurements. a) The leaf section will be dried after taking it out of ethanol, then nail varnish will be applied to a specific area and left to dry. Tape will be used to take a negative impression of the leaf surface using the nail varnish copy. The tape, with the varnish impression, will be laid on a microscope slide and stomatal anatomy will be analysed. b) A longitudinal section of the leaf will be cut and put through a clearing protocol using ethanol and bleach, then stained with Fast Green and Safraning. The leaf section will then be laid on a microscope for analysis of vein features, as the stains infiltrate the xylem and highlight the veins. c) Leaf longitudinal section will cut and embedded in wax, before using a microtome to cut very thin section. Those sections will also be laid on a slide and imaged under microscope for leaf anatomical analysis.
2. Measurement of RNA expression. Frozen leaf samples will be finely ground, then total RNA extracted using Purezol. Aliquots of RNA are then treated with DNase and using an RNA kit.

Opportunity for Skill Development

1. Analysis of leaf anatomy and molecular makeup is an important and sought-after lab technique in modern plant and agricultural sciences.
2. Gain an understanding of how to design and conduct scientific experiments as well as the analysis of data and interpretation of results.
3. Gain experience in the requirements to perform and the day to day life in a research lab aiming to find solutions for modern challenges in food security and climate change.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

No specific prerequisites.

Project 65: Hawkesbury Urban Forest Experiment – testing a diversity array of trees to Western Sydney's hot dry conditions

Supervisor(s): Paul Rymer - p.rymer@westernsydney.edu.au
Principal Supervisor

Mohomah Kibria - m.kibria3@westernsydney.edu.au
Second Supervisor

This project is in partnership with the **Department of Planning, Industry and Environment**

Project Description

As Sydney's urban population grows, it is critically important green infrastructure is developed to provide essential socio-economic and environmental services. Urban forests are vulnerable to climate change, however, and recent drought and heatwave events have resulted in significant failures. Currently, tree species selection and management options are uncertain. This proposal will establish a state-of-the-art research facility and demonstration site "Hawkesbury Urban Forest Experiment" designed to test the growth and performance of 48 diverse native and exotic tree species from a wide range of climate-origins. This site will be ideally located in Hawkesbury City Council, where maximum temperatures are regularly 6-10°C higher and rainfall is 400-500mm less than eastern Sydney, thus providing a proxy for future expected climate changes in Greater Sydney. The incorporation of controlled irrigation will investigate how supplemental water might enable a diverse array of trees to survive and thrive into the future. The outcomes of the project include a unique demonstration site of urban tree species growing side-by-side designed to inform species selection under climate change. It will engage and train policy makers, land managers and practitioners in the delivery of urban greening and cooling strategies to improve the liveability of communities across Greater Sydney and eastern Australia.

Project Aims

The overall aim of the project is to investigate species' climate-origin as a predictor of tree growth and performance to inform species selection in developing green and resilient urban forest in Western Sydney into the future. The water requirements of urban tree species will be characterised to inform the management requirements and ability for irrigation to enable greater diversity in Western Sydney's urban forests. The specific objectives are (1) estimate growth rates, (2) measure functional traits, and (3) performance and stress in 48 trees species established in the Hawkesbury Urban Forest Experiment. Analyses will be conducted to determine the differences among species, watering treatments, and the interaction to provide insights into species local adaptation to climate.

Project Methods

The summer scholars will work closely with researcher in the Hawkesbury Institute for the Environment to quantify plant growth, performance and functional traits. Plant performance will be measured using a range of integrated approaches including physiological estimates of plant stress during hot summer conditions. Students will be encouraged to take part in pre-dawn measures of plant water potential, gas exchange measures of photosynthesis and stomatal conductance, along with fluorometric measures of stress. Branch material will be harvested to estimate wood density and specific leaf area along with other functional traits known to response to environmental conditions.

Opportunity for Skill Development

The student will gain skill and experience in understanding how an experiment is set, learning to measure plant performance in terms of different measures across the plants as well as being methodical in managing big quantities of plants in experiments. Also, the student will learn to measure morphological traits as specific leaf area and wood density that are pretty useful in determine ecological function and strategy. The student will learn novel tools in physiological research which we hope will motivate its curiosity and research expertise. Finally, the student will be guided to analyse the data and obtain graphics and statistics that led to make and support solid conclusions.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

First and most important thing is motivation and commitment with the experiment. Understanding of basic plant biology, good organisation and analytical skills. Accuracy in taking measurements.

Project 66: Tree tolerance to drought and heat extremes in urban trees

Supervisor(s): Renee Prokopavicius - r.prokopavicius@westernsydney.edu.au
Principal Supervisor

Mark Tjoelker - M.Tjoelker@westernsydney.edu.au
Second Supervisor

Project Description

The average climate across Australia is projected to warm 2.8 to 5.1 °C by 2090 with an increased frequency and intensity of heatwaves. There are currently targeted initiatives to increase green tree canopy in many Australian cities to help create cooler, more sustainable cities for the future. The urban forest provides shade and evaporative cooling (via transpiration), a combination that can reduce air temperatures by 2–8 °C. However, recent extreme drought and heatwaves have shown that some widely planted tree species are unlikely to continue to function under higher mean and extreme temperatures. Resilience of the urban flora will depend on appropriate species selection based on their capacity to grow and transpire under increasingly hot and dry climate extremes.

This project will test plant tolerance to drought and heat extremes using the unique whole-tree chambers at the Hawkesbury Institute for the Environment. These outdoor, sunlit chambers can accommodate 9-m tall trees and enable implementation of experimental drought and heatwaves under field conditions. Are species from warm climates closer to their thermal limits than those from cool climates? Does high heat tolerance rely on plant water access through deep rooting and/or a supply of irrigation? This project is supported by two Australian Research Council Discovery grants and involves measurements of leaf temperature and leaf thermal tolerance in tree species. Filling these knowledge gaps will greatly enhance our ability to create resilient urban forests in a warmer world.

Project Aims

Plant heat stress tolerance can be influenced by watering, heatwave intensity, as well as other plant leaf traits that may differ among species of contrasting climate of origin. The aims for this student summer scholar project are to:

- Gain experience in using scientific equipment to collect research data
- Measure leaf temperature and thermal tolerance across plant species of different origin and growth form
- Determine differences in stress tolerance among plant species
- Learn how to summarise and report research-based findings.

Project Methods

This project uses plant Eco physiological techniques that will involve leaf-level measurements in the whole-tree chambers on the Hawkesbury campus. Leaf temperatures will be measured using a thermal imaging camera and an infrared thermometer. Heat tolerance of leaves will be determined using a pulse-amplitude modulated fluorometer (MINI-PAM-II/B). The supervised student will be contributing to a larger bulk of data collection to be led and undertaken by a team of researchers at HIE, including the project supervisors (Dr Prokopavicius and Prof Tjoelker).

Opportunity for Skill Development

This project will introduce the student to the fields of plant physiology and urban ecology. The student will develop skills using several pieces of scientific equipment, as well as skills for proper data collection, organisation, hypothesis-testing and analysis:

- Learn the importance of precise, accurate measurements following a standard protocol
- Learn to work with scientific equipment
- Learn how to collate, statistically analyse and interpret primary data
- Learn to present data appropriately and effectively.

The student will learn to function independently after receiving proper guidance, while contributing to a larger research team and experiment.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

Student candidates should be able and willing to work outdoors in the field at a supervised research site on the Hawkesbury Campus in Richmond. Student candidates will be trained in and expected to follow safe-work practices and relevant COVID guidelines & restrictions. Work will sometimes involve brief inclement conditions (hot days during summer) and changes in schedule.

Project 67: Altering the primary carboxylase of C4 photosynthesis to improve crop production.

Supervisor(s): Robert Sharwood - r.sharwood@westernsydney.edu.au
Principal Supervisor

Oula Ghannoum - o.ghannoum@westernsydney.edu.au
Second Supervisor

Project Description

Extreme climate events and decline in available water are threatening crop yield and quality necessitating new solutions to ensure optimal water use within future food systems. This project seeks to identify natural biochemical solutions within C4 plants belonging to the Panicum tribe that confer improved responses to carbon assimilation and plant productivity under future climates. The project will harness latest biotechnologies to interrogate the natural diversity in the catalytic properties of the primary carboxylase of C4 photosynthesis - phosphoenolpyruvate carboxylase (PEPC). PEPC is responsible for the CO2 supply to the carbon concentrating mechanism (CCM) which in turn enables the substantial rise of CO2 within bundle sheath chloroplasts to saturate Rubisco with CO2. A major requirement of the CCM is to have PEPC activity 5 – 7 times greater than that of Rubisco- and to select the isoforms that harbour superior catalytic properties and are less inhibited by malate. Therefore, this project will interrogate the natural catalytic diversity of PEPC enzymes across the biochemical subtypes of C4 photosynthesis, which are determined by the major decarboxylase enzyme present i.e. NAD-ME, NADP-ME and PCK. The project builds on preliminary success of expressing recombinant PEPC isoforms in E. coli that will provide the necessary framework to determine critical amino acid residues responsible for catalytic diversity. The outcome of this project is to provide new biochemical solutions to improve the catalytic responses of PEPC that will ultimately utilised to improve carbon assimilation and water use through altering PEPC in key C4 crops such as maize and sorghum.

Project Aims

The core fundamental question addressed by this project will be: What are the key amino acids that underpin the catalytic variability observed in PEPC? This question will be addressed by two specific research aims:

1. Clone and express PEPC isoforms with domain swaps into E.coli expression constructs to purify PEPC protein, and
2. Examine the diversity of PEPC catalysis and metabolic regulation of activity across different biochemical subtypes.

Project Methods

Aim 1: Clone and express PEPC isoforms with domain swaps into E.coli expression constructs to purify PEPC protein

Utilising RNA sequencing data generated by CI Sharwood, the coding sequences of the photosynthetic PEPC isoform from representative biochemical subtypes (NADP-ME, NAD-ME, PEPCK) within the Panicum C4 grass tribe were identified and subsequently synthesised for optimal expression in E. coli. Alignment of the PEPC amino sequences revealed subtype differences in the malate/aspartate binding region which is adjacent to catalytic regions (Figure 1A).

The three representative isoforms including the domain swaps have been incorporated into the Ubiquitin E. coli protein fusion system developed by CI Sharwood (Figure 1B). This system enables cleavage of ubiquitin that allows for high purity production of recombinant protein (Figure 1C). Building on the preliminary success of expressing PEPC proteins (Figure 1C), an additional two constructs will be designed from each subtype and synthesised prior to the start of the summer student. The summer student will participate in cloning of the new genes into the pHUE vector for expression in E. coli (see above). In addition, the student will make chimers of PEPC isoforms to further interrogate the functional consequence of these amino acid deletion and substitutions. The prospective student will express and purify the PEPC proteins using developed protocols by CI Sharwood to create a library of enzymes for catalytic testing.

Aim 2: Examine the diversity of PEPC catalysis and metabolic regulation of activity across different biochemical subtypes. Working with CI Sharwood, the summer student will measure the PEPC catalytic properties following the assay protocols from Sharwood et al., 2016 JXB. This will enable testing of the differences in catalytic speed and inhibition of PEPC isoforms by metabolites oxaloacetate and malate.

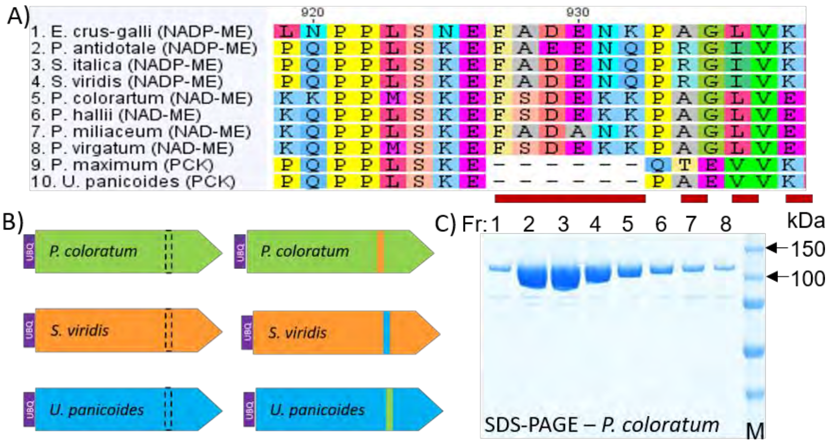

Figure 1. Expression of Panicum PEPC isoforms in E.coli. (A) Amino acid alignments of Panicum PEPC amino acids has revealed a subtype dependent diversity between isoforms. (B) PEPC genes synthesised from three Panicum species and preliminary domain swaps to test the impact of the 6 amino acid deletion. (C) The three selected PEPC enzymes were successfully expressed in E.coli; the cleavage of ubiquitin was successful and the enzyme was purified further by SEC (shown for P. coloratum).

Opportunity for Skill Development

In addition to contributing data for publication, the proposed student will gain valuable experience in:

1. Recombinant DNA cloning and protein expression in E. coli;
2. Protein separation techniques including size exclusion chromatography (SEC) and affinity chromatography;
3. Protein electrophoresis and quantification of proteins using imaging techniques;
4. Enzyme assays using a UV-VIS spectrophotometer and associated data analyses.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

Students would be required to have completed 300850 (Autumn 2021) Advanced Cell Biology, have experience in the use of pipettes and able to use Excel for data plotting and analysis.

Project 68: Uncovering heat tolerant metabolic enzymes within wild cotton species.

Supervisor(s): Robert Sharwood - r.sharwood@westernsydney.edu.au
Principal Supervisor

Warren Conaty
Second Supervisor, External Partner, **CSIRO Cotton Breeding Institute**

Project Description

Future cotton production in Australia is seriously threatened by rising annual temperatures, and subsequently more frequent extreme heatwaves and drought conditions, as evidenced in the past few years. These extreme climate events are detrimental to the cotton community and the local economy. Therefore, there is substantial demand to increase heat- and drought-stress tolerance in elite cotton cultivars to minimise the impact on the Australian cotton industry. Greater reductions in precipitation and availability of irrigation water will generate further restrictions on the rainfed and irrigated cotton industry in the very near future. Photosynthetic performance and resilience are traits that have not attained their full potential in cotton. Multiple studies have documented that photosynthetic enhancement should be a major focus of future cultivar development programs to sustain and improve crop yields in future challenging climates. This overall aim is to generate new, in-depth knowledge around abiotic stress physiology and plant biochemistry, particularly by focusing on diverse cotton germplasm that are likely to possess adaptations to extreme climates. The project dovetails with the recently funded CRDC project to CI Sharwood – Climate-proof Cotton (UWS2201). The target of this project is Rubisco activase (RCA) which is an important regulatory protein involved in maintaining the active state of Rubisco, but its sensitivity to high temperature leads to deactivation of Rubisco. The project will screen cotton RCA isoforms from diverse cotton germplasm originating from hot and dry environments. Identified thermotolerant versions of RCA will be utilised in future pre-breeding efforts to generate a new cotton prototype to cope with extreme climates.

Project Aims

Increasing ambient air temperatures is a serious challenge facing Australia's world-leading cotton industry. To build resilience to future hot and dry climates we are focussing on improving the performance of cotton photosynthesis at elevated temperatures by identifying thermotolerant isoforms of Rubisco activase. This enzyme modulates Rubisco carbon fixation activity and is thermolabile, which causes decreased carbon assimilation at leaf temperatures above 35oC. Therefore this project aims to identify thermotolerant isoforms of Rubisco activase from wild species of cotton that originate from hot dry climates using latest synthetic biotechnologies. The specific research aims of the project include:

1. Clone and recombinantly express and purify Rubisco activase isoforms within in E. coli for catalytic analyses; and
2. Identify Rubisco activase isoforms that display improved catalytic activity above 35oC through measuring ATPase activity.

Project Methods

CI Sharwood has developed the ubiquitin fusion protein system for recombinant expression of plant proteins in E. coli. This system ensures absolute purity of Rubisco activase protein with the correct mature N-terminal amino acid residue. Four Rubisco activase β-isoforms from wild species have already been identified and synthesised by Twist Biosciences. The student will clone each of the isoforms into pHUE for recombinant expression and purification. Improved thermotolerance will be determined by the student measuring ATPase activity from 10 - 37°C using an NADH-coupled assay system. Variants exhibiting a higher thermal optimum of ATPase activity will be selected as ATPase activity coincides with the ability to activate and maintain cotton Rubisco activity at higher temperatures. Amino acid sequences will be interrogated to identify amino acid residues conferring enhanced thermotolerance.

Opportunity for Skill Development

In addition to contributing data for publication, the proposed student will gain valuable experience in:

1. Recombinant DNA cloning and protein expression in E. coli;
2. Protein separation techniques including size exclusion chromatography (SEC) and affinity chromatography;
3. Protein electrophoresis and quantification of proteins using imaging techniques;
4. Enzyme assays using a UV-VIS spectrophotometer and associated data analyses.

Students are required to have the following skills/meet the following pre-requisite(s) to apply

Students would be required to have completed 300850 (Autumn 2021) Advanced Cell Biology, have experience in the use of pipettes and able to use Excel for data plotting and analysis.