Potential projects on plant cell walls at the Edinburgh Cell Wall Group 2023

PROJECTS OPEN TO VISITING STUDENTS, POSTDOCS, AND SABBATICAL ACADEMICS

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CONTENTS

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English language requirements: The University of Edinburgh has strict English language requirements for visiting students. See note at end.

GENERAL INTRODUCTION

CONTEXT

The cells of all land-plants, from mosses to the tallest trees, have robust cell walls, important both to the life of the plant and to human society as a sustainable resource. Our interest in cell walls comes from both these angles:

(1) In the living plant, cell walls dictate the shape and size of cells (and ultimately of the whole plant); they act as barriers to pathogens; they sequester toxic metals in polluted soils; they 'glue' neighbouring cells (giving roots, stems, leaves and fruits their unique

textures); and they issue 'messages' called oligosaccharins.

(2) Plant cell walls are the world's most abundant organic material, much of it currently in the form of low-value by-products of food crops, but potentially processable into valuable liquid biofuels or chemical raw materials, in place of petrochemicals. Most plant biomass occurs in thick secondary cell walls, but for good reasons our work focuses on the thin primary cell wall (**PCW**), which dictates cell size. Although too thin to be a *bulk* resource, the PCW is pivotal in fixing the area of the cell surface, on whose inner face a much bulkier secondary wall may later be deposited. The secondary wall can never exceed the PCW in area; in this sense biomass deposition is limited by the PCW, and understanding the PCW is a key to controlling plant biomass production.

Recent papers from The Edinburgh Cell Wall Group (ECWG)

2016

202 O.B. Airianah, R.A.M. Vreeburg, S.C. Fry (2016) Pectic polysaccharides are attacked by hydroxyl radicals in ripening fruit: evidence from a fluorescent fingerprinting method. *Annals of Botany*, **117**, 441–455.

203 D. Chormova, S.C. Fry (2016). Boron bridging of rhamnogalacturonan-II is promoted *in vitro* by cationic chaperones, including polyhistidine and wall glycoproteins. *New Phytologist*, **209**, 241–251.

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PROJECT 1
Why do plants require boron, and how do they attach it to pectic polysaccharides?

Boron (B) might not immediately sound like the most exciting of elements, conjuring up images of 'boring' and 'moron'! In this project, we will try to demonstrate that first impressions can be misleading. Unlike most organisms, plants have an absolute requirement for the element B. Although B deficiency can be overcome by fertilisers, excess B in some soils is an intractable agricultural problem. Why do plants require B, and what is the basis of its toxicity when present in excess? Boron's major role in plants is to cross-link a cell-wall polysaccharide called rhamnogalacturonan-II (RG-II). However, why this is important to the cell is unclear. We will study the effect of different B concentrations on (a) the cross-linking of RG-II *in vivo* and (b) aspects of cell and tissue structure such as growth and cell–cell adhesion, looking for cause/effect relationships.

RG-II is a complex pectic domain in plant primary cell walls. *In vivo*, most RG-II domains are covalently dimerised via borate diester bridges, essential for correct cell-wall assembly, but the dimerisation of pure RG-II monomers by boric acid (H_3BO_3) *in vitro* is extremely slow.

Very recently, we have discovered that certain plant arabinogalactan-proteins (AGPs) act as enzymes, catalysing the reaction

$$2\ \text{RG-II} + H_3BO_3 \rightarrow \text{RG-II–B–RG-II}$$

thus serving as RG-II borate diesterase enzymes. It is of interest that the Enzyme Commission does not currently list any enzymes acting on boron compounds (https://iubmb.qmul.ac.uk/enzyme/EC3/).

In more detail, the reaction can be represented as (Fig. 1):

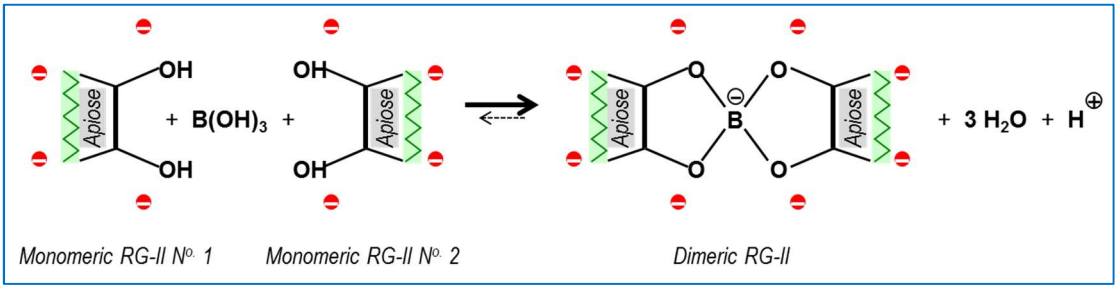

Figure 1. The essential reaction involved in boron bridging of RG-II.

Part of the neutral apiose residue of side-chain A (in each of two monomeric RG-II molecules) is shown. The charges of its four near-neighbouring anionic sugar residues are rendered as red circles (two α-GalA residues in the RG-II backbone plus an α-GalA and a β-GalA residue attached to the rhamnose adjacent to the apiose). Monomeric RG-II has a total of about 11 additional anionic sugar residues, giving it a net charge of about –14 at the pH (4.8) used in our experiments. It is evident that the close approach of two RG-II monomers for boron bridging requires the overcoming of considerable electrostatic repulsion, and dimerisation introduces an additional negative charge on the previously neutral boron atom.

We tested cationic peptide fragments of several *Arabidopsis thaliana* AGPs as candidate 'boron enzymes'. Fragments of AGP17, 18, 19 and 31 were effective, typically at ~25 μg/ml (9–19 μM), promoting the boron bridging of 16–20 μM monomeric RG-II at pH 4.8 *in vitro*. Native AGP31 glycoprotein was also effective, and hexahistidine was moderately so. All these catalysts interacted *reversibly* with RG-II and were not consumed during the

reaction; thus they acted catalytically, and may constitute the first reported boron-acting enzyme activity, an **RG-II borate diesterase**. Many of the peptide chaperones became less effective catalysts at higher concentration, which we interpret as due to the formation of RG-II–peptide complexes with a net positive charge, as mutually repulsive as negatively charged pure RG-II molecules. The four unique AGPs studied here may serve an enzymic role in the living plant cell, acting on RG-II within Golgi cisternae and/or in the apoplast after secretion. In this way, RG-II and specific AGPs may contribute to cell-wall assembly and hence plant cell expansion and development.

Ca^{2+} ions also promote RG-II dimerisation, but far less effectively than the non-biological metal ion, Pb^{2+}. We will investigate whether Ca2+ is rendered more effective if applied to RG-II in synergy with 'boron enzyme' AGPs.

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[2] R.A. Begum, S.C. Fry (2022) Boron bridging of rhamnogalacturonan-II in *Rosa* and arabidopsis cell cultures occurs mainly in the endo-membrane system and continues at a reduced rate after secretion. *Annals of Botany* in press. DOI: 10.1093/aob/mcac119

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Methods:
Wet lab work.
Growing plant cell-suspension culture.
Extracting RG-II from the cell walls.
Chromatography or electrophoresis to separate cross-linked from non-cross-linked RG-II.
Microscopy to evaluate cell size and cell–cell adhesion.

PROJECT 2
Understanding strawberry cell walls as a guide to breeding future-proof fruit genotypes

Rationale. This project comprises novel research that is both of fundamental interest and has industrial applicability. The softening of ripening fruit involves dramatic changes in cell-wall structure. In many species, softening is important because it makes fruit attractive to consumers. On the other hand, excessive softening is detrimental, causing wastage on the supermarket shelf and making crops more vulnerable to pathogens. Controlling the softening process, e.g. in strawberries, would cause less waste when UK crops peak; imported crops (e.g. from Southern Europe and Morocco in winter) could go further; and opportunities would be raised for summer UK exports. Slow-softening fruits would bring less disease and longer picking intervals.

Preliminary data from the Fry lab (Edinburgh) is elucidating novel mechanisms by which fruits soften. This work draws on many years' experience of cell-wall biochemistry. The project may involve collaboration with Edward Vinson Ltd. (Kent), commercial fruit breeders, who have a molecular breeding pipeline — in strawberry among other fruits.

Proposed new research
Available genotypes differ in firmness: for example, recently developed commercial genotypes *versus* 'heritage' varieties. We will explore the relationship between their genetics and their cell-wall processes. At Edinburgh, we will invent convenient new tools for screening cell-wall 'softening markers', including:

- vitamin C secretion and turnover;
- hydroxyl radicals that non-enzymically attack cell walls *in vivo*;
- solubilisation and partial degradation of pectins;
- specific pectin-targeting enzyme activities and/or action in living fruit—
    - pectin methylesterase
    - rhamnogalacturonan lyase
    - pectate lyase
    - endopolygalacturonase.

Research role: To explore the physiological/biochemical processes occurring in softening strawberry cell walls. Devising novel, simplified assays for these processes suitable for routine use by plant breeders. This study will employ the most appropriate types of chromatography and electrophoresis, with detailed practical guidance at Edinburgh.

Later, depending on initial findings and personal motivation, we will devise the best ways forward to fully characterise the relevant biological processes. This may include *in-vivo* radiolabelling in Fry's lab, and NMR spectroscopy plus mass spectrometry in the School of Chemistry of the University of Edinburgh.

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The research will help to develop convenient new tools for screening cell-wall 'softening markers', including:

- vitamin C (ascorbate) secretion and turnover [ascorbate has long been known to be present in fruits, but evidence for its secretion into the cell wall is a more recent advance];
- hydroxyl radicals non-enzymically attacking cell-wall polysaccharides *in vivo* [recently shown to be produced and to act in ripening fruit]
- solubilisation and partial degradation of pectic polysaccharides [based on direct studies of pectin chemistry rather than on deductions from gene expression];
- specific pectic enzyme activity and/or action in living fruit (these activities can be assayed by published methods which, however, are labour intensive; our project will develop new, high-throughput tools for assaying them)—
    - pectin methylesterase
    - pectate lyase
    - rhamnogalacturonan lyase
    - endopolygalacturonase

These new tools are aimed at providing a novel 'omics' approach to monitoring the softening of ripening fruits.

PROJECT 3

Developing a novel method for assaying the plant cell wall-loosening protein, expansin

Expansins (not to be confused with extensins!) are proteins that loosen plant cell walls, thus enabling plant growth. Expansins cleave hydrogen-bonds between cell-wall polysaccharides, especially cellulose, though they do not catalyse any change in the polysaccharides' covalent structure. Currently they can only be assayed by a tedious method in which cell wall samples are physically stretched and their 'creep' (irreversible elongation) is measured.

In this project we will pursue our unpublished data which offer the methodology for creating, for the first time, a high-throughput quantitative assay for expansin activity based on expansins' ability to open up the crystalline structure of cellulose fibrils to the external solution. Expansins cleave hydrogen-bonds between polysaccharides, thus loosening the plant cell wall, but do not affect the covalent structure.

We will attempt to devise, for the first time, simplified tests for expansin activity. Two approaches will be tried:

(a) based on expansin's ability to make cellulose more accessible to a cellulose-attacking enzyme, HTG (hetero-trans-β-glucanase);

(b) based on expansin's ability to release labelled oligosaccharides from a sample of solid cellulose to which they have been hydrogen-bonded.

Devising a convenient, high-throughput, sensitive and quantitative assay would be valuable from several perspectives associated with food security. For example:
・screening for novel herbicides that target growth-essential plant expansins.
・investigating the production of expansins by nematodes (or other plant pathogens), and screening for novel compounds that target these effector proteins.

During the project we will

- isolate plant expansins,
- isolate a bacterial expansin heterologously produced in *E. coli*,
- attempt to generate plant expansins heterologously in the yeast *Pichia*,
- manufacture (radioactively or fluorescently labelled) polysaccharide and oligosaccharide substrates,
- work up a preliminary low-throughput methodology for detecting expansin action *in vitro*,
- develop the preliminary technique into a high-throughput version.

We will use techniques including

- plant cell wall analysis,
- protein purification,
- radiolabelling, and
- enzyme assays.

Prof. Fry has many years' experience of cell wall biochemistry and has developed novel assays for various cell-wall components, including boron cross-links, XET (xyloglucan endotransglucosylase) activity, and hydroxyl-radical-attacked polysaccharides.

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PROJECT 4
In-vivo formation of isodityrosine and its higher homologues (pulcherosine and di-isodityrosine) in the glycoprotein extensin

Extensins (not to be confused with expansins!) are hydroxyproline-rich structural glycoproteins of the plant cell wall. They are impossible to solubilise intact, indicating strong covalent cross-linking within the wall architecture. The cross-linking was proposed to be due to tyrosine–tyrosine dimers, forming 'isodityrosine' [Fry, 1982]. However, it remains unknown whether these dimers are inter-protein bridges rather than intra-protein loops.

The project will test this by novel *in-vivo* isotopic feeding experiments. Cultured plant cells will be grown in standard 'light' medium (containing ordinary sugar), then suddenly transferred into 'heavy' medium containing ^{13}C-sugar. Thereafter, 'old' and 'young' extensin chains will contain the isotopes ^{12}C and ^{13}C respectively. After further incubation, we will hydrolyse the extensin and purify its constituent isodityrosine. Mass spectrometry will then be used to discover whether most of the isodityrosine (Tyr-Tyr) molecules contain both isotopes (as expected if forming inter-protein cross-links between old and young chains), or a single isotope (some isodityrosine molecules with ~100% ^{12}C and others with ~100% ^{13}C; as expected for intra-protein loops).

The project will also explore several other unknowns about the biology of extensins. The laboratory has techniques to hand to answer the following questions:

- When during the 'career' of an extensin molecule do cross-links based on isodityrosine and the corresponding tetramer di-isodityrosine form?
- What forces extensins to dimerise their tyrosine residues (in plants *in vivo*) in the form of isodityrosine instead of dityrosine, the dimer which forms predominantly *in vitro* (and in animals *in vivo*).
- How do extensins interact with neighbouring pectin molecules, especially rhamnogalacturonan-II (RG-II)? Does RG-II dictate isodityrosine vs dityrosine production? And does extensin direct the creation of boron bridges between RG-II molecules?

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PROJECT 5
New cell-wall polysaccharides and enzymes that remodel them in charophytic algae

ALGAL ANCESTORS OF LAND PLANTS: All land plants, from mosses and liverworts to flowering plants, comprise a single group known as 'embryophytes', which evolved from a common ancestor. Genetic fingerprinting shows that the embryophytes' closest living relatives are a group of freshwater green algae called charophytes. It is uncertain which charophyte group (Charales or Coleochaetales) is closest-related to the land plants.

Very little is known about the exciting events that occurred roughly 460,000,000 years ago, when certain charophytes evolved into the first (liverwort-like) embryophytes. Leaving the water and adapting to terrestrial life would have necessitated numerous biochemical and anatomical changes. Among the most important (for withstanding desiccation and lack of buoyancy) must have been those involving cell walls.

IMPORTANCE OF PLANT CELL WALLS: The cell wall is the tough, outermost layer of a plant cell. Primary walls (those of young, growing cells) serve numerous vital roles, especially that of constraining the rate and direction of cell expansion, and thus ultimately controlling the growth of the whole plant.

ABUNDANT KNOWLEDGE OF LAND-PLANT CELL WALLS: Land-plant primary walls consist mainly of sugar-based polymers (polysaccharides), which are categorised into cellulose, pectins and hemicelluloses, and whose chemical structures have been studied in detail. Hemicellulose molecules are chain-like 'tethers' within the cell wall, constraining cell expansion. Major hemicelluloses in most land-plant primary walls are xyloglucans, xylans and mannans. However, grasses, cereals and, as we recently discovered, horsetails (Equisetum) possess an additional hemicellulose: mixed-linkage glucan (MLG).

MINIMAL KNOWLEDGE OF CHAROPHYTE CELL WALLS: In contrast, little is known about the cell-wall composition of charophytic algae. They contain cellulose and pectins, but their hemicelluloses are ill-characterised. They contain no detectable xyloglucan, a marked difference from land plants. MLG was also undetectable. Much remains to be discovered about charophyte cell-walls: information that would guide our understanding of the earliest land plants.

CELL-WALL RE-MODELLING ENZYMES IN LAND PLANTS: Plant cell walls are not inert 'boxes': they contain various enzymes that re-model the wall, adjusting its properties during development. One re-modelling enzyme, found in all land plants, is xyloglucan endotransglucosylase (XET) activity. It cuts one xyloglucan chain (donor substrate) and joins a portion of it on to another, similar chain (acceptor substrate). This re-modelling process probably contributes to cell-wall assembly and loosening, facilitating cell expansion.

In Equisetum, but no other land plants, we recently discovered a novel enzyme, comparable to XET but preferentially using MLG as donor substrate and xyloglucan as acceptor. This enzyme was termed MXE (MLG:xyloglucan endotransglucosylase). Since Equisetum possesses both xyloglucan and MLG, MXE should be capable of re-modelling these.

CELL-WALL RE-MODELLING ENZYMES IN CHAROPHYTES: We also found that charophytes contain both enzyme activities (XET and MXE), which may thus be primordial cell-wall features, already present while the earliest embryophytes were colonising the land. This discovery was intriguing because the enzymes' target polysaccharides (xyloglucan and MLG) appear to be absent from charophytes. Why do charophytes possess these enzymes if there is nothing for them to do?

OBJECTIVES

We aim to discover primordial features of land-plant cell walls by characterising the

walls of their closest living relatives the charophytes. The curiosity-driven question is: "what cell-wall components were seconded into new roles when aquatic algae evolved into embryophytes, adapted to terrestrial life?" We will provide the first detailed analysis of the cell-wall polysaccharides of extant charophytes, enabling a comparison with land plants. We will also explore the newly discovered enzymes that re-model these polysaccharides in algae.

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PROJECT 6
Novel xyloglucan-modifying enzyme activities that re-structure the angiosperm primary cell wall

Our lab has discovered several new xyloglucan-modifying enzymes. One was '**XET**', first reported in 1989 (Baydoun & Fry). Since then we have found others, including **MXE** (Fry et al., 2008), trans-α-xylosidase (TaX; Franková & Fry, 2011) and others (Franková & Fry, 2012a,b). This project focuses on **TaX** and four other new xyloglucan-modifying enzymes. We wish to discover exactly what reactions these enzymes catalyse. Importantly, we will test whether these enzymes actually modify the built-in xyloglucans of the PCWs of living plants [*a priori*, one could argue that the enzymes may not act in this way, but only under artificial conditions in the test-tube]. We will develop simplified tests for the new enzymes, enabling 'high-throughput' screening. We will use the simplified assays to help us purify the new enzymes, thus leading to discovery of their genes.

Since 1989, much interest has centred on the biological and commercial significance of XET, which 'cuts and pastes' xyloglucan chains, helping to build and re-model the PCW (Franková & Fry, 2013). XET can only do its job if the xyloglucan has the right structure, so it may be that the new enzymes alter XET action; we will test this hypothesis directly. Finally, we will test whether the new enzymes modulate the activity of the xyloglucan-derived 'messages' mentioned above.

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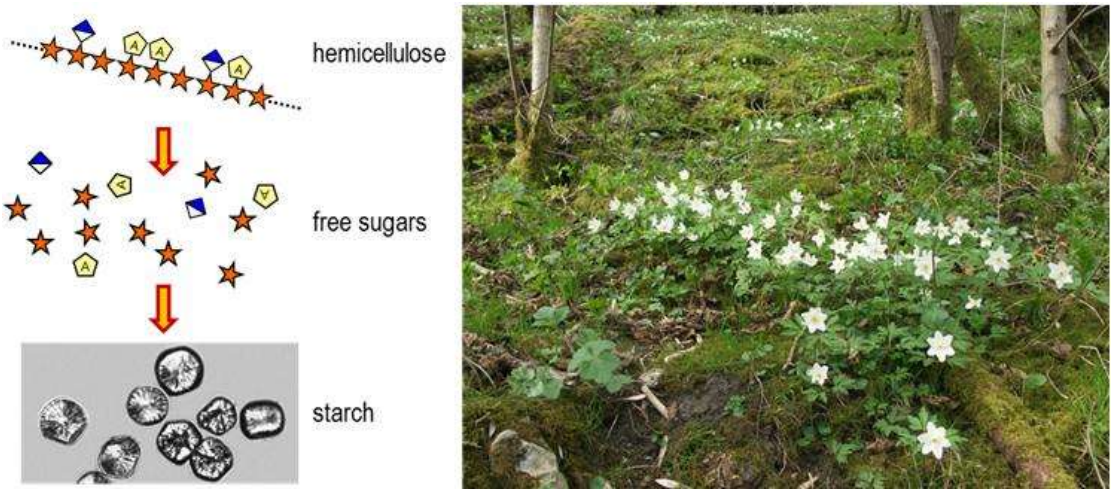
PROJECT 7

Adaptation to shade: Do shaded saplings and forest-floor herbs salvage carbon from their cell-wall polysaccharides?



Forest-floor plants mobilise structural polysaccharides, enhancing survival in deep shade

Summary

Saplings and forest-floor herbs may be carbon-deficient owing to shading. They salvage C from starch prior to leaf abscission. But when/where do they also salvage C from structural polysaccharides, their major organic resource?

Project background

Carbon (C)-starved plants salvage C reserves, e.g. from stored starch. This project explores the emerging idea [2,3] that structural polysaccharides e.g. hemicelluloses [1] are also mobilised during severe C starvation and the released sugars used for respiration and biosynthesis. Hemicelluloses are often ~25% of a plant's dry weight, and the biosphere's 2nd most abundant organic material. Thus, C-salvaging from leaf hemicellulose prior to abscission can appreciably augment a starved plant's C status and impact the global C cycle.

Ecologically relevant instances of C starvation are

* Deciduous saplings in an existing forest.

* Herbs (e.g. wood anemones) on the forest floor, where there is only transiently (in spring) enough light. Such herbs should salvage maximal leaf C for underground storage.

* Herbivore-attacked plants, left with insufficient foliage.

The project will provide solid new data on hemicellulose mobilisation in starved plants' mature leaves, and discover when, where and to what extent this occurs. We will also test whether cellulose and pectin are salvaged.

The project will make an important new contribution to plant ecophysiology.

Research questions

To what extent do the leaves of young deciduous trees and forest-floor herbs carry out the reported hemicellulose mobilisation prior to abscission?

Is the mobilisation of foliar starch and hemicellulose greater in carbon-starved trees and herbs (e.g. heavily shaded or partially defoliated)?

Which particular hemicelluloses are most subject to mobilisation (xyloglucan, xylan, mannan)?

Are other leaf structural polysaccharides (cellulose, pectin) also appreciably mobilised in carbon-starved trees and herbs?

What is the fate (translocation/metabolism) of monosaccharides released from foliar hemicelluloses?

Methodology

Growing deciduous saplings (oak, beech) and forest-floor herbs (bluebell, wood anemone) under standard glasshouse conditions. Tracking saplings in natural habitats. Checking for disease-free status. Monitoring changes in their foliar dry weight during senescence and at abscission. Learning the techniques of polysaccharide analysis to quantify foliar hemicelluloses (xyloglucans, xylans and mannans separately), pectin, cellulose and starch. Recording this quantification in leaves during senescence and after abscission.

Repeating the polysaccharide quantification in leaves of heavily shaded and partially defoliated (versus control) plants. The senescing leaves will be harvested at timed intervals during summer (woodland herbs) and during autumn (saplings), freeze-dried, and analysed for polysaccharides when time permits.

Determining the major monosaccharides lost from hemicelluloses during senescence (chromatography and colorimetric analysis). Exploring the fate (translocation and metabolism) of these monosaccharides by use of radioisotopic tracers.

Training

Learning the techniques of starch and cell-wall polysaccharide fractionation and their quantification.

Learning the techniques of identifying and quantifying the monosaccharide residues of hemicelluloses.

Participating in the University's radiation protection course.

Taking responsibility for housekeeping of a selection of the laboratory's communal equipment.

Presenting research group seminars (3 per year) and posters at international meetings.

Attending weekly one-to-one meetings with Prof. Fry to troubleshoot and discuss plans.

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PROJECT 8
Vitamin C breakdown in plants and 'smoothies'

Ascorbate and dehydroascorbic acid (DHA) both serve as vitamin C in the human diet. They are interconvertible within the cells of both animals and plants. However, a cell's vitamin C pool can be diminished by various irreversible pathways which start out from DHA. This is of obvious significance in governing the vitamin content of living plants and also of plant-derived foods and beverages (e.g. smoothies). Some of the pathways are enzyme-catalysed, though many of them occur without enzymes to catalyse the reactions. Our laboratory has made several recent contributions to understanding these routes that lead to vitamin loss, both in plants and under bio-mimicking conditions in vitro (see several references below). The present project will further define the metabolic fate of vitamin C and the biological and nutritional consequences of these pathways.

There are two competing routes that irreversibly diminish vitamin C levels by consuming DHA:

(a) Oxidative degradation of DHA to oxalyl threonate esters, oxalate and threonate. This route is promoted under conditions of oxidative stress, for example when reactive oxygen species (ROS) are present. Downstream of route (a), the oxalyl threonates can attach their oxalyl groups to carbohydrates (Dewhirst & Fry, 2018a), including cell-wall polysaccharides, and the threonate can be oxidised to tartrate.

(b) Non-oxidative hydrolysis of DHA to diketogulonate (DKG). This route is promoted at pH values above about 6 and when ROS are not present at high concentration. Downstream of route (b), the DKG can undergo several further reactions of oxidative, reductive and non-redox nature (Kärkönen et al., 2017; Dewhirst & Fry, 2018b).

The majority of these pathways are of unknown biological significance, and a major aim of the present project is to explore their significance.

Specific topics <u>to be addressed in this project</u> include:

- Defining the effect of ozone on ascorbate, DHA and DKG. These ascorbate-derivatives are thought to 'mop up' ozone from atmospheric pollution, but the by-products thus formed are unknown. We will continue our work on ROS-driven oxidation of ascorbate and its derivatives (several references listed below), adding ozone to the list of ROS already studied.
- Defining when and where oxalyl groups from route (a) are attached to cell-wall polysaccharides (Dewhirst & Fry, 2018a) and whether there is evidence that wall-bound oxalate has effects on the properties of the cell wall.
- Defining the pathways of vitamin C loss in smoothies (e.g. 'liquidised' spinach leaves), and helping to define factors that could be recommended to minimise such loss.
- Testing how much of a plant cell's total ascorbate is secreted through the plasma membrane into the apoplast (aqueous solution that permeates the cell wall). This is significant because the cell wall contains an enzyme, ascorbate oxidase, that converts ascorbate to DHA (Green & Fry, 2005).
- Defining the routes via which vitamin C is lost when spinach leaves intended for salad use are commercially washed. The mechanical aggitation suffered by such leaves has been noted to diminish ascorbate levels (Dewhirst et al., xxxx), but the mechanism for this remains unknown.
- Testing whether routes (a) and (b) are triggered to some extent by enzymes. For example, the hydrolysis of DHA to DKG can occur non-enzymically but in addition, there is early preliminary evidence that 'DHA hydrolase' enzymes exist, both plants (Tewari & Krishnan, 1960) and in animals (Kagawa Y et al., 1961), and this project will investigate this hypothesis as a contribution to understanding the control of vitamin C levels *in vivo*. We will extract plant

enzymes and test their ability to catalyse DHA → DKG hydrolysis. We will also look for novel enzyme activities that catalyse the oxidation of DHA and/or DKG.

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PROJECT 9
Xyloglucan-derived oligosaccharins and their hormone-like action on growth and development in plant cell and tissue cultures

CONTEXT

Oligosaccharins are specific fragments of plant cell wall polysaccharides and have potent 'hormone-like' effects on growth and development (McDougall & Fry, 1988; Fry et al., 1993; Aldington & Fry, 1993; Gonzalez-Perez et al., 2012). Plant cell cultures will be used in this project to study the effects of oligosaccharins.

The primary cell wall (PCW) is mainly composed of long-chain sugar-polymers of 3 main classes: cellulose, hemicelluloses and pectins. We will focus on oligosaccharins produced from hemicelluloses, especially one type called xyloglucan — a molecular chain that is thought to adhere to cellulose fibres and tether them, constraining cell expansion. Certain oligosaccharins have been discovered to be spontaneously produced by plant cell cultures (McDougall & Fry, 1991; Smith & Fry 1999).

AIMS AND OBJECTIVES

Xyloglucan can be cleaved to generate oligosaccharides, some of which exhibit potent 'hormone-like' biological effects at very low concentrations on plants, plant cell cultures and plant tissue cultures. Plant cell cultures are an excellent system for studying the control of plant cell growth, and will be exploited in the present project. In addition, callus cultures will be used to test for oligosaccharin effects on organogenesis.

Our lab has discovered several new xyloglucan-modifying enzymes (for review, see Franková & Fry, 2013). One was '**XET**', first reported in 1989 (Baydoun & Fry). Since then we have found others, including **MXE** (Fry et al., 2008), trans-α-xylosidase (TaX; Franková & Fry, 2011) and others (Franková & Fry, 2012a,b). These enzymes act to modify xyloglucan-derived oligosaccharins, potentially altering their 'hormone-like' biological activities. This project focuses on the enzyme **TaX** and four other new xyloglucan-modifying enzymes. We wish to discover exactly what reactions these enzymes catalyse when acting on xyloglucan-derived oligosaccharins. We will develop simplified tests for the new enzymes, enabling 'high-throughput' screening. We will then use the simplified assays to help us purify the new enzymes.

We will test whether these new enzymes modulate the bio-activity of the xyloglucan-derived 'messages' (oligosaccharins) mentioned above.

Besides commercial applications, the work will guide and inform future fundamental studies of plant cell growth and development, re-writing an important chapter in plant cell biology.

TECHNICAL SUMMARY

Xyloglucan, a polysaccharide in the PCWs of all land plants, is thought to tether the cellulosic microfibrils and restrain cell expansion, and also to generate growth regulators ('oligosaccharins'). Over the years, our lab has discovered several novel xyloglucan-modifying enzyme activities, including xyloglucan endotransglucosylase (XET; Baydoun & Fry, 1989), MXE (Fry et al., 2008) and trans-α-xylosidase (TaX; Franková & Fry, 2011, 2012b). Recently (Franková & Fry, 2012a), we added trans-β-galactosidase (**TbG**) and a probable xyloglucan-acting galactose oxidase (**GaO**). Xyloglucan-modifying enzymes may remodel both PCW architecture and oligosaccharins, controlling cell expansion.

This project will characterise TaX, TbG and GaO activities in vitro, defining their substrate specificities and reaction-products. Equally important, we will determine the effect of the new enzymes on the 'hormone-like' properties of xyloglucan-oligosaccharides.

The project will provide important, missing, fundamental information about the biology and chemistry of reactions that modify xyloglucan, an architectural component of the primary cell walls in all land plants and a source of potent signalling molecules known as oligosaccharins. The significance of the results is not confined to any particular plant genus or family, but applies across the board to land plants in general.

The work exploits this lab's recent discovery and partial characterisation of xyloglucan-acting transglycosidase activities, and is guided by progress on xyloglucan studies being made in other labs worldwide. It is also guided by our own progress towards the industrial exploitation of novel cell-wall enzymes. It is predicted to lead to the refinement and better definition of oligosaccharins with which scientists can manipulate the growth and development of plant cell and tissue cultures.

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PROJECT 10
Towards an understanding of the role(s) of silicon in cell walls of higher plants

Silica is a major component of the cell walls of certain land-plants, especially members of the Poaceae (grasses and cereals) and the Equisetaceae (horsetails), and also in certain specialised cells of various other plants e.g. the stinging hairs of the Urticaceae (nettles) [5]. It is present at much lower concentrations in the walls of other plants (e.g. cucumber, tomato and generally all dicots) growing in the same soils, showing that silica deposition is under the plants' genetic control. Silica provides a degree of physical strength to cell walls (helping to explain why it is possible to cut one's finger on a blade of grass), and acting as a grazing deterrent. For example, it has been demonstrated by field observations that prairie voles avoid feeding on grass with higher silica content and that the growth of small phloem-feeding herbivores is stunted if there is too much silica in their diet.

Uniquely, this project will employ for the first time in plant biochemistry the radioactive isotope of silicon, ^{32}Si. This isotope has properties (energy, etc.) somewhat comparable with those of the widely used carbon isotope, ^{14}C, and will alow us for the first time to trace the transport of Si within the plant and its covalent and non-covalent binding to organic components [6,7] of the plant, especially cell-wall polysaccharides.

An ability to manipulate the silica content of plants would be beneficial for our ability to control the nutritional value of fodder and susceptibility of crops to pathogen attack. Such ability would be strongly promoted by an understanding of the fundamental mechanisms controlling silica deposition in plants, and this question is explored in the present proposal. Strategies that would become feasible for future workers armed with such knowledge would include genetic manipulation, conventional plant breeding, and optimised agricultural practice (including application of silicon-containing fertilisers). In the present research proposal we will investigate aspects of the chemistry of silicon (in some form) in selected land-plants including *Equisetum*, barley and maize. The experimental design for the project involves chemical, biochemical and biological studies on the cell walls of plants grown hydroponically and of cell cultures of maize and barley. We will investigate how plants take up from the soil soluble compounds containing the element silicon (Si), and then move them into the stems and leaves, where the soluble compounds are converted into deposits of insoluble silica (which is chemically similar to glass), which strengthen the cell walls. We will also discover to what extent small silicon-containing groups are linked to the polymers (polysaccharides) of which plant cell walls are composed. We will explore the possibility that silicon-compounds serve as cross-links between cell-wall polysaccharides, helping the plant to 'build' its cell walls. The project requires chemical, biochemical and biological expertise in order to tackle such problems as 'the identity of silicon-containing biomolecules within the plant cell wall', and 'translocation of the element from the roots through the plant to the final point of silica deposition'.

Plants make insoluble silica from soluble silicic acid, which they find in the soil. However, most plants (e.g. dicots) accumulate little silica, even when growing in the same soil as silica-rich cereals. Plants noted for having a very high silica content in their cell walls include horsetails (fern-relatives of the genus *Equisetum*), nettles (dicots of the genus *Urtica*) and grasses and cereals (order Poales). This project will focus on *Equisetum* and *Urtica*, together with, for comparison, a low-silica dicot: cucumber (*Cucurbita*).

We recently discovered that horsetails share with grasses and cereals [and some of their very close relatives] one unique chemical feature: they possess a very unusual polysaccharide (a hemicellulose called 'mixed-linkage glucan' or MLG) in their cell walls, as well as enzymes that act on it [3,4]. We will test the hypothesis that MLG plays a major role in steering the process of silica deposition, such that silica deposited in the presence of MLG (in *Equisetum*) differs from that deposited in the cell walls of *Urtica* (which lacks MLG).

Equisetum is the sole surviving genus of the Equisetopsida, a formerly diverse and dominant class of fern-relatives that probably diverged from their closest living relatives about >370 million years ago. *Equisetum* species are of little commercial significance but are masters of silicification that can

probably teach us much about the mechanisms of this process [their gritty texture, due to silica, accounts for their American name and former use as 'scouring rushes']. Their silica is also unique in terms of its solubility, which may be of therapeutic benefit. Of reports in the literature, the mean silicon content of the genus *Equisetum* is an extremely high 45.9 g kg^{-1}; the mean for *Urtica* is 13.1 g kg^{-1}; and that for all dicots is 4.2 g kg^{-1}.

We will isolate the cell walls from these three plant species (grown with and without a good supply of silicic acid from which they can make silica), and then digest them with enzymes so that the insoluble cell-wall polysaccharides are cleaved into small, soluble 'fragments'. These fragments will be sorted on chromatography columns, and those that carry the element silicon will be studied in detail. In some experiments, the plants will first have been fed radioactive silicon (^{32}Si), which will act as a 'label' to facilitate the detection of the substances of interest.

The use of ^{31}Si is a unique feature of this project, as this isotope has very rarely been used in biological studies.

In addition, we will mix pure cell-wall polysaccharides (especially MLG but also others; from Equisetum and *Urtica*) with soluble silicic acid and study how they bond to each other (and/or form insoluble silica). In some experiments, we will 'force' silica deposition by partial drying, mimicking the effect of water evaporation from a plant's shoot system. The 'artificial' silicon–carbohydrate complexes thus formed will be analysed in detail by chromatography.

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PROJECT 11
Specific chemical agents to define the roles of wall enzymes in the control of plant cell growth: discovery of potential new herbicides

The overall aims are to devise and apply novel methods [e.g. based on Ref. 1] for screening the effects of xenobiotics (small molecules which do not naturally occur in plants) on cell wall-related enzymes, and to discover suitable (effective, specific, non-toxic) inhibitors of these enzymes. Such inhibitors could be used to provide new information on the specific biological roles of individual cell-wall enzyme activities and wall polysaccharides. In addition, since the pectin/hemicellulose/cellulose cell wall is unique to land-plants, inhibitors that interfere with the action of the relevant wall enzymes could yield valuable leads for potential novel herbicides. Unusually, in this project, the target enzymes are external to the plasma membrane and can therefore be reached by hydrophilic inhibitors.

Individual objectives are:
(i) devising novel screens for wall-related enzyme activities [this work has been partly completed — Ref. 2];
(ii) assembling a collection of xenobiotics [this work has been partly completed — Ref. 2];
(iii) testing the collection and identifying specific inhibitors of wall enzymes;
(iv) defining the effectiveness, specificity and phytotoxicity of inhibitors;
(v) defining the effects of specific, non-toxic inhibitors on plant physiology, especially cell expansion.

Intended Outcomes:
Academic tool: Provision of a selection of xenobiotics with which we can probe the biological roles of wall-localised enzymes speculated to be involved in 'wall loosening' and the mechanism of plant cell expansion.
Agro/industrial exploitation: Discovery of leads for potential novel herbicides.

Specific disciplines to be used:

- Extraction of enzymes from vegetables (from supermarket).
- Preparation of labelled substrates (radioactive and/or fluorescent) for screening the enzymes.
- Devising simple, reliable, effective high-throughput screens for selected enzyme activities, and their inhibition by xenobiotics.
- Testing the toxicity of the xenobiotics on plant cell cultures.
- Skill sets: confidence/familiarity with methods of wet biochemistry.

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LANGUAGE REQUIREMENTS, APPROXIMATE COSTS and HOW TO APPLY

English language requirement for students

The University of Edinburgh has strict English language requirements for visiting research (V_{Res}) students from many countries (for exceptions, see list below). The minimum requirement for the College of Science and Engineering is

- IELTS 6.5 (with 6.0 in each section) or
- TOEFL 580 (with 55 in each section & 4.0 in Test of Written English TWE) or
- TOEFL 237 in CBT (with 21 in each section & 4.0 in TWE) or
- TOEFL 88 in IBT (with 19 in each section) or
- CPE Grade B or CAE Grade A.

All overseas visiting students are required to provide formal evidence of competency in English language **except** for those students who come from English-speaking countries including Australia, Botswana, Canada, Fiji, Gambia, Ghana, Jamaica, Kenya, Lesotho, Liberia, Malawi, New Zealand, Nigeria, Papua New Guinea. Sierra Leone, South Africa, Swaziland, Tanzania, Uganda, United States of America, West Indies, Zambia and Zimbabwe.

Students who have undertaken degree level education in the medium of English in a country where English is not the first language will be required to provide a formal test result.

All tests submitted must have been taken within 24 months of the date of proposed admission to The University of Edinburgh.

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- Visiting students will be charged a VRes tuition fee, approximately £810 per annum.

In addition, you need to consider:

- Cost of living: approx £15,500 / year.
- Laboratory expenses: minimum £2,000 / year

For more details, see
https://www.ed.ac.uk/students/new-students/finances/student-living-costs

How to apply

Visiting Students: If you are considering joining **ANY** of these projects, please make an informal enquiry to Professor Stephen Fry, describing which project is of interest and why it interests you, and explaining what aspects of your previous studies you feel will qualify you for the work envisaged.