1. Title of Case Study: The Molecular Architecture of Plant Cell Walls

2. Grant Reference Number: Contract: PR140003

3. One sentence summary: The plant cell wall assembles through precise induced molecular interactions as evidenced by high-field solid-state NMR.

4. One paragraph summary:

Dupree and co-workers have made use of the improved resolution obtained at high magnetic field to investigate cell wall architecture in the model plant *Arabidopsis* grown in air containing ^{13}C-enriched CO_2. The aim of this study was to understand the molecular basis of plant cell wall properties, such as recalcitrance, which hinders the use of plant biomass for renewable energy by inhibiting the conversion into fermentable sugars. Both wild-type *Arabidopsis* and cellulose-defective mutants were investigated using multi-dimensional ^{13}C MAS NMR. Specifically, the induced folding of the xylan onto the surface of cellulose fibrils was discovered. This work is significant because pilot biofuel production processes involve the use of energy-intensive pre-treatments and the addition of large amounts of enzymes to remove xylan from cellulose, and to digest the xylan and cellulose to fermentable sugars.

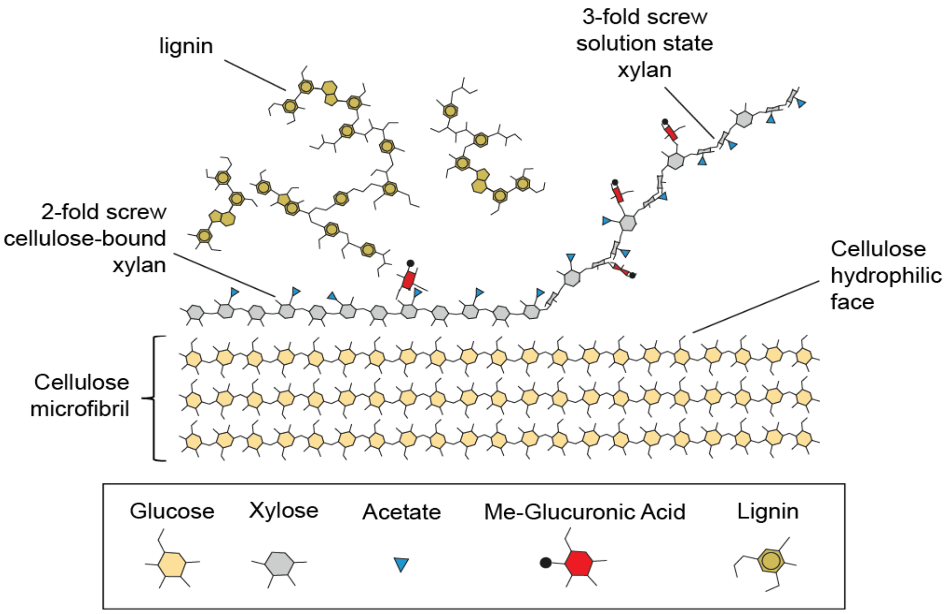
5. Key outputs in bullet points:

- *Demonstration of a new theory of plant cell wall assembly*
- *Discoveries will allow increased use and improved applications of woody materials for building construction, energy, materials and food*
- *Training, notably in solid-state NMR of plant cell walls, of a postdoctoral worker Dr Thomas Simmons, currently a BBSRC/Royal Society of Edinburgh Enterprise Fellow*
- *New collaboration with Dr Eduardo DeAzevedo, University of Sao Paolo, Brazil, in NMR studies of plant materials.*

6. Main body text

Materials from plants have been used by humans over millennia for their food, to feed animals, for clothing, and for building construction as timber. As the largest available resource of renewable carbon on the planet, in the future plants are likely to provide sustainable ways to avoid fossil fuel use. However, increased exploitation of this plant cell wall biomass is hampered by our ignorance of the molecular basis for its properties such as strength and digestibility. Cellulose is the main component of the plant cell wall material, and it is present as long, strong fibrils, set like steel reinforcement rods within a mixture of other components. These other components include long chains of other sugars, such as xylan. Xylan is the most prevalent non-cellulosic polysaccharide and it binds tightly to cellulose microfibrils. The atomic-scale nature of this interaction remains unclear, despite the likely importance in providing strength to timber and in preventing digestion of woody plants.

In this project our aim was to study plant cell walls and to find out how cellulose binds to xylan. We developed techniques to study intact plant cell walls with solid-state NMR spectroscopy, which gave us information on the shape of the components and also the distance between them. The very high resolution obtained at the 850 MHz NMR Facility enabled us to distinguish many of the different components in the cell wall. We showed (*Nat. Comm*. **7**, 13902 (2016)) that the majority of xylan, which forms a flexible chain in solution, flattens into a ribbon to bind intimately to cellulose microfibrils in the cell wall. This supports the model shown in the figure.



Cellulose: xylan interaction model. The figure shows the new model of cellulose: xylan interactions in which non-cellulose-bound xylan exists in its characteristic flexible shape called a "3-fold helical screw", while binding of xylan to the cellulose surface induces xylan to change shape into a flat ribbon, the "2-fold helical screw".

The discovery that induced polysaccharide shape underlies plant cell wall assembly provides new principles to understand woody cell wall properties. This method and discovery will now lead to development of better processes for paper and packaging production, and also in improvements to digestion of plant materials for animal feed and bioenergy. Since timber is used widely for building construction, we believe this model will also allow development of better methods for wood modification and preservation.

Other relevant references: Dupree et al., *Biochemistry* **54** 2335-2345 (2015) & Zhang et al., *Nat. Comm*. **7** 11656 (2016).

7. Names of key academics and any collaborators:

Professor Paul Dupree (University of Cambridge)
Professor Ray Dupree (University of Warwick)
Professor Steven P. Brown (University of Warwick)
Dr Eduardo Azevedo (University of Sao Paolo)
Professor Staffan Persson (University of Melbourne)

8. Sources of significant sponsorship (if applicable):

Contract for the High Field Solid State Nuclear Magnetic Resonance Facility (EPSRC)
BBSRC (The BBSRC Sustainable Bioenergy Cell Wall Sugars Programme)

9. Who should we contact for more information?

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