Raman Spectroscopy – A Novel Approach to Diagnosing Ash Dieback and Oak Powdery Mildew

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Background:

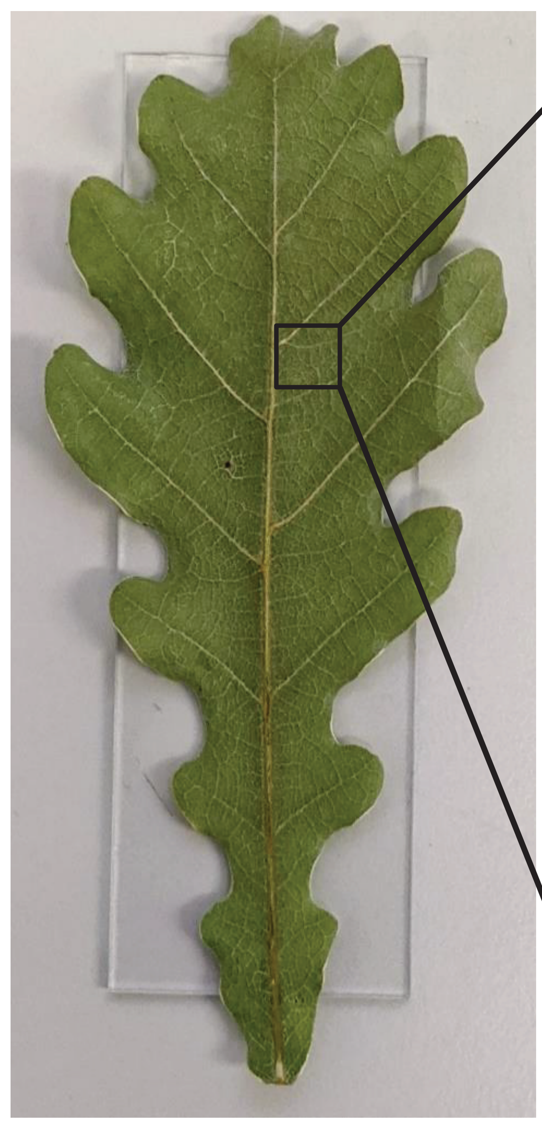
Ash dieback and oak powdery mildew are fungal diseases affecting ash and oak trees across the UK, threatening young trees. Raman spectroscopy is a nondestructive analysis technique which can provide insights into the early stages of infections. This method may provide a tool for early diagnosis, allowing more effective management of these diseases.

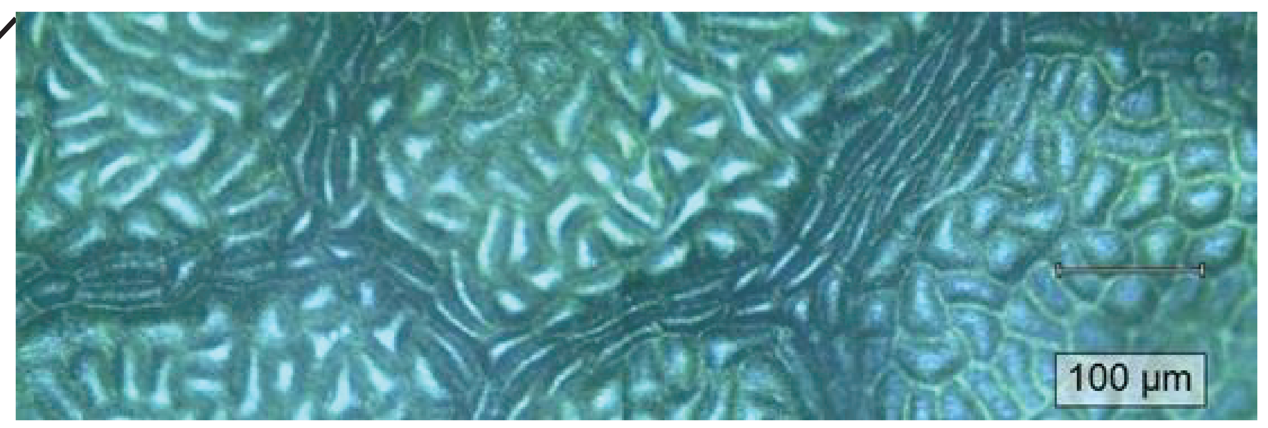
Objectives:

To develop procedures for the analysis of non-vein, secondary vein and primary vein tissues. These procedures will be used to produce models of infection by comparing healthy and infected leaf spectra for each tissue type. Saplings will be grown in both ambient (aCO_2) and elevated CO_2 environments (eCO_2) to compare the infection models in order to identify any differences. Finally, a handheld Raman spectrometer will be designed to identify these changes in the field.

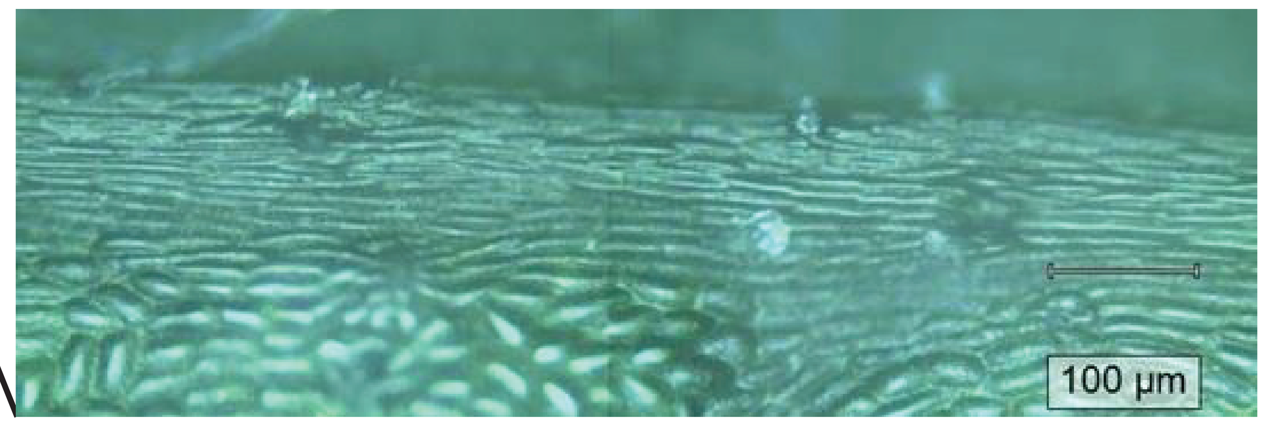
Method:

Healthy and infected leaves were mounted onto glass slides and placed into the Raman microscope. At x20 magnification, areas of non-vein, secondary vein and primary vein tissue were measured using an 830nm laser.



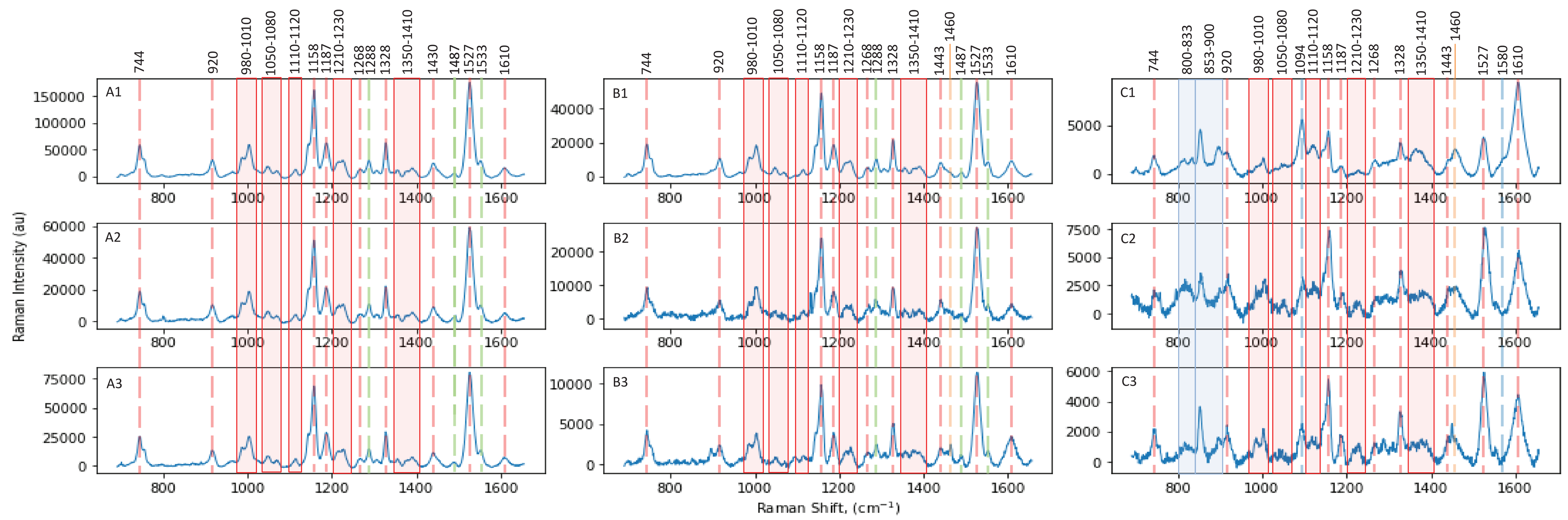


Non-Vein & Secondary Vein Tissue



Primary Vein Tissue

Preliminary Results:



Key: A - Non-vein | B - Secondary vein | C - Primary vein | 1 - Healthy | 2 - Infected Symptomatic | 3 - Infected Asymptomatic

Raman Peak Assignments:

Key	
Colour	**Tissue Types**
	Primary Vein
	Non-Vein and Secondary Vein
	Primary and Secondary Vein
	All

Peak Wavenumber (cm^{-1})	Tentative Assignment	Peak Wavenumber (cm^{-1})	Tentative Assignment
744	Pectin [1]	1268	Lignin [3] and Carotenoids [6]
800-833	Aliphatics [2]	1288	Aliphatics [2]
853-900	Pectin [1]	1328	Cellulose, Lignin [3] and Chlorophyll [6]
920	Cellulose and Lignin [3]	1350-1410	Aliphatics [2]
980-1010	Carotenoids [3] and Phenylalanine [4]	1443	Aliphatics [2]
1050-1080	Cellulose, Lignin [3]	1460	Cuticle Triterpenoids [2]
1094	Cellulose [5]	1487	Aliphatics [2]
1110-1120	Cellulose and Carbohydrates [3]	1527	Carotenoids [3]
1158	Carbohydrates, Cellulose [3] and Carotenoids [6]	1553	Chlorophyll [6]
1187	Lignin [3] and Chlorophyll [6]	1580	Unknown
1210-1230	Aliphatics, Xylan [3] and Carotenoids [6]	1610	Phenolics and Lignin [6]

Future Work:

- To use a computational algorithm to distinguish between the tissue type spectra
- To identify biomarkers of ash dieback and oak powdery mildew by analysing infected leaves
- To measure eCO_2 leaves to identify any differences between aCO_2 and eCO_2 spectra
- To develop a handheld Raman spectrometer to identify biomarkers and thus diagnose these diseases in the field

References:

References: [1] A. Synytsya, "Fourier transform Raman and infrared spectroscopy of pectins," Carbohydrate Polymers, vol. 54, no. 1, pp. 97-106, 2003, doi: 10.1016/s0144-8617(03)00158-9. [2] M. M. L. Yu, H. G. Schulze, R. Jetter, M. W. Blades, and R. F. B. Turner, "Raman Microspectroscopic Analysis of Triterpenoids Found in Plant Cuticles," Applied Spectroscopy, vol. 61, no. 1, pp. 32-37, 2007, doi: 10.1366/000370207779701352. [3] C. Farber, M. Shires, K. Ong, D. Byrne, and D. Kurouski, "Raman spectroscopy as an early detection tool for rose rosette infection," Planta, vol. 250, no. 4, pp. 1247-1254, Oct 2019, doi: 10.1007/s00425-019-03216-0. [4] D. Kurouski, R. P. Van Duyne, and I. K. Lednev, "Exploring the structure and formation mechanism of amyloid fibrils by Raman spectroscopy: a review," Analyst, vol. 140, no. 15, pp. 4967-80, Aug 7 2015, doi: 10.1039/c5an00342c. [5] H. G. M. Edwards, D. W. Farwell, and D. Webster, "FT Raman microscopy of untreated natural plant fibres," Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 53, no. 13, pp. 2383-2392, 1997/11/01/ 1997, doi: https://doi.org/10.1016/S1386-1425(97)00178-9. [6] L. Mandrile et al., "Nondestructive Raman Spectroscopy as a Tool for Early Detection and Discrimination of the Infection of Tomato Plants by Two Economically Important Viruses," Anal Chem, vol. 91, no. 14, pp. 9025-9031, Jul 16 2019, doi: 10.1021/acs.analchem.9b01323

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