Electronic Supporting Information

Spectrochemical analysis of sycamore (*Acer pseudoplatanus*) leaves for environmental health monitoring

James Ord[a,b], *Holly J. Butler*[b], *Martin R. McAinsh*[b]*, *and Francis L. Martin*[b,c]*

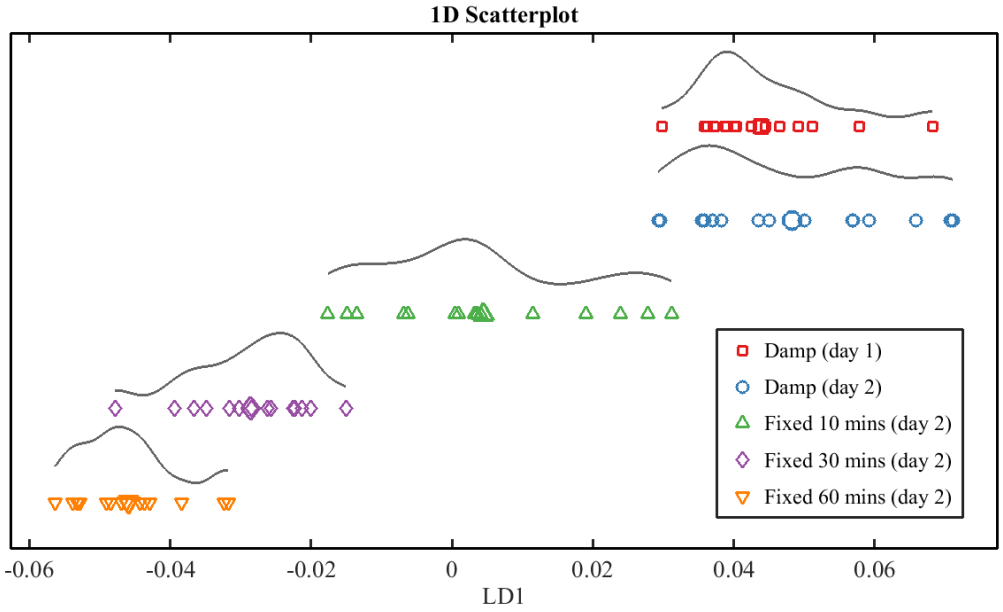
[a]Department of Animal and Plant Science, University of Sheffield, Sheffield S10 2TN, UK; [b]Centre for Biophotonics, Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK; [c]School of Pharmacy and Biomedical Sciences, University of Central Lancashire, Preston PR1 2HE, UK

*Corresponding authors: Prof Francis L Martin / Dr Martin R McAinsh; *Email:* f.martin@lancaster.ac.uk; m.mcainsh@lancaster.ac.uk

Summary

- **Number of pages: 4**
- **Number of Figures: 2**
- **Number of Tables: 2**

A)



One-way ANOVA	LD1
Damp (day 1) vs. Damp (day 2)	$P > 0.05$
Damp (day 1) vs. Fixed, 10 mins (day 2)	$P < 0.001$
Damp (day 1) vs. Fixed, 30 mins (day 2)	$P < 0.001$
Damp (day 1) vs. Fixed, 60 mins (day 2)	$P < 0.001$

B)

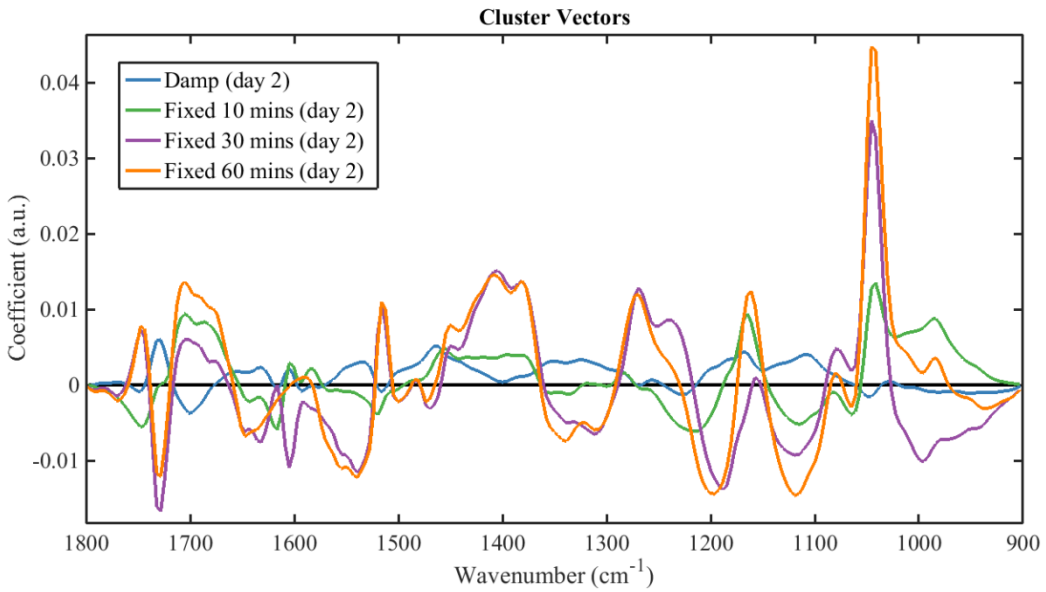


Figure S1 (A) One-D PCA-LDA scores plot derived from *A. pseudoplatanus* leaf tissue under different fixation conditions. Leaves (5×) were fixed in 70% ethanol for 10, 30 or 60 min, left to dry for 2 h and wrapped in aluminium foil. A further 5× leaves were not fixed and instead stored in zip-lock bags containing damp cotton wool. Spectra were acquired from fixed leaves ≈24 h after collection. Spectra were acquired from damp leaves on the day of collection, and ≈24 h after collection. Spectra (3×) were acquired per leaf. The damp condition had no significant effect on LD1 after 24 h, but ethanol at all three immersion times had a highly significant effect on LD1 as determined by one-way ANOVA (P <0.001). **(B)** Cluster vectors plot by PCA-LDA indicating wavenumber basis for segregation after fixation of *A. pseudoplatanus* leaf tissue with 70% ethanol, plus non-fixed leaves, 24 h after collection. Each class is compared with non-fixed leaves on the day of collection. The magnitude of the cluster vector peak or trough is proportional to the extent of biochemical alteration compared to non-fixed leaves on the day of collection.

Table S1 Average Fv/Fm readings taken from the leaves of mature *A. pseudoplatanus* trees at the three main field sites. Student's T-tests revealed no significant difference between polluted sites and Reference.

Site	Average Fv/Fm	Number of readings	Significance (vs. ref. site)
Site 1	0.81	20	
Site 2	0.82	25	N/S
Site 3	0.81	18	N/S

(N/S) No significance ($p > 0.05$)

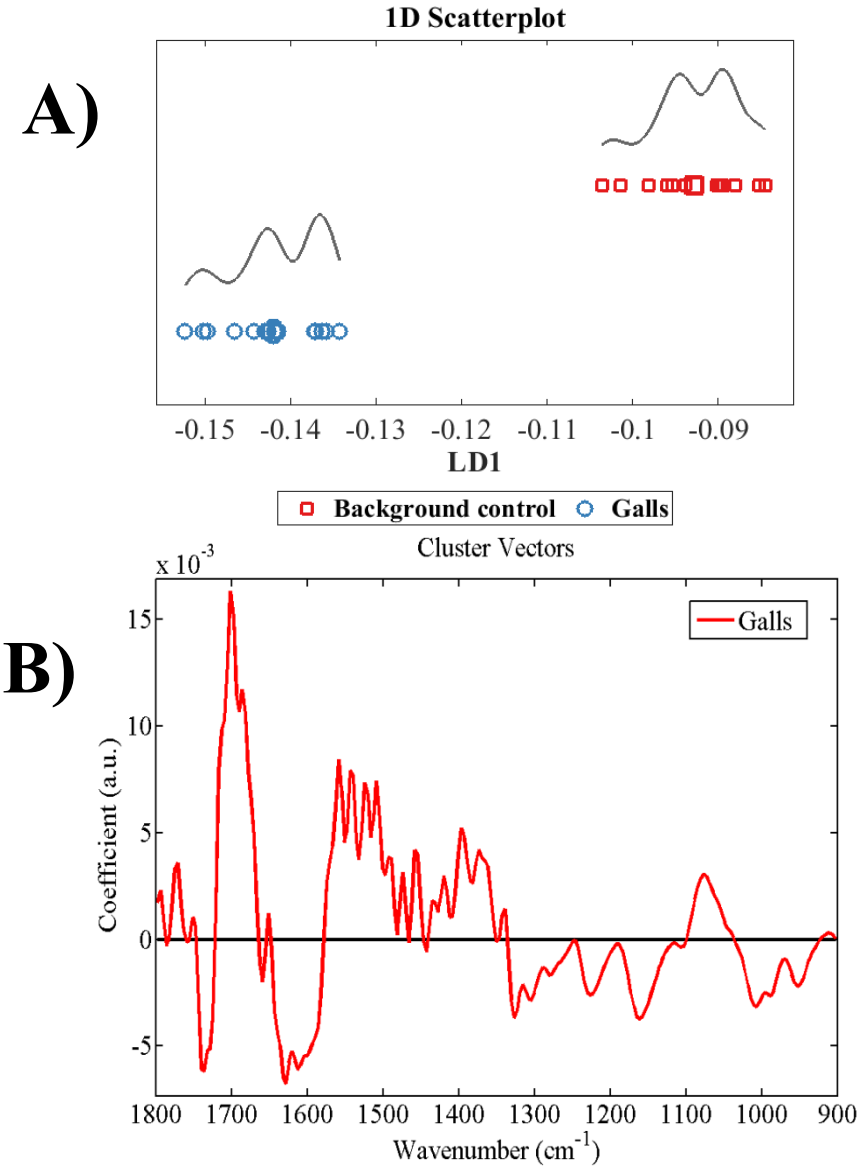


Figure S2 (A) One-D PCA-LDA scores plot of spectra derived from *A. pseudoplatanus* leaf tissue afflicted by galls of the mite *Artacris macrorhynchus*, compared to the background control leaf tissue (displaying no obvious affliction. Segregation in LD1 space was highly significant (P <0.001) as determined by Student's T-test. **(B)** Cluster vectors plot by PCA-LDA indicating wavenumber basis for segregation in *A. pseudoplatanus* leaf tissue afflicted by galls, compared with the background control leaf tissue (origin).

Table S2 Top six discriminating wavenumbers (in descending order) identified by cluster vectors, associated with differences in *A. pseudoplatanus* leaves afflicted by leaf galls of the mite *Artacris macrorhynchus*, in relation to background control leaves. Tentative chemical assignments from Movasaghi *et al* (2008), Schulz and Baranska (2006), and Stuart (2004).

Wavenumber (cm^{-1})	Tentative assignment(s)	Response (relative B.C.)
1701	Lipid; fatty acid esters	Increase
1585	Amide I	Decline
1520	Amide II	Increase
1632	Amide I; Pectin	Decline
1458	Protein; $\delta_{as}CH_3$	Increase
1169	Carbohydrate	Decline