UNDERSTANDING PLANT RESPONSES TO PATHOGEN STARTS WITH A GOOD PROTOCOL

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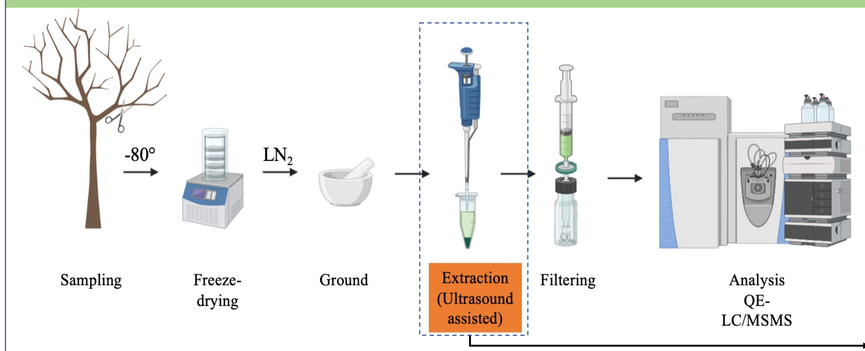
Introduction:

Woody infecting pathogens, such as bacterial ash canker[1], are increasing in importance in the UK. To date, little is known regarding the plant responses towards the pathogen. Plant metabolomics is a powerful way to study and understand plant responses to the environment allowing the identification of for example diseased biomarkers[2]. **However, there is no optimised method for tree extraction**[3]. Factors such as chemical diversity is important as the more chemical that can be recovered will avoid bias during the analysis and selection of **biological markers to understand plant responses to biotic and abiotic stressors**. Studies has shown that chemical diversity and yield of extracts from different plant organs and species can be solvent and temperature dependant[3,4]. Thus, it has been **strongly recommended to conduct experiments optimising extraction protocols** in order to achieve the highest chemical diversity and yield[2,3,4].

Methods: experimental design and workflow



Temperature

- 4° C
- 20° C
- 50° C

Cycles

Solvent

Aim: To test the effects of temperature, number of cycles and polarity of the solvent on metabolites extracted on woody tissue using an untargeted metabolomics approach.

Healthy ash trees (*Fraxinus excelsior*) from University of Birmingham campus and conduced a fully factorial design; testing all the possible combinations of the factors (18 total) to test the extraction solvent.

Results

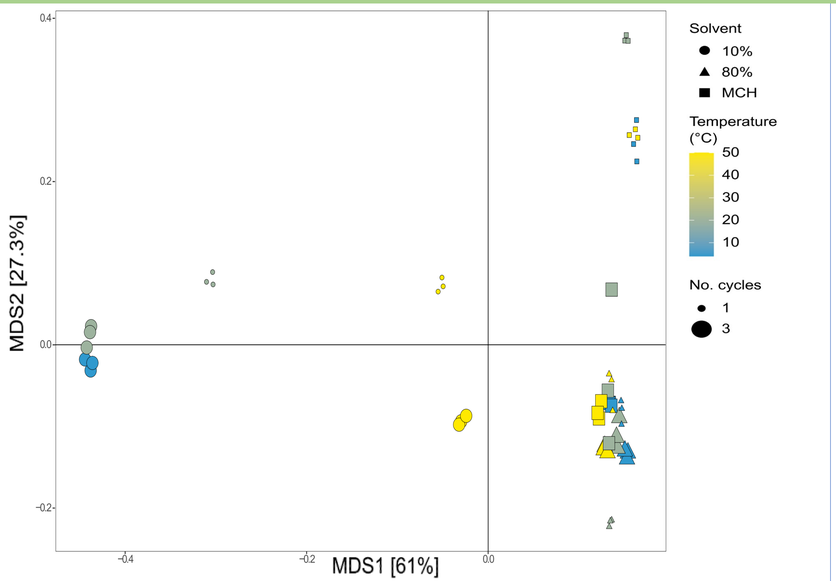


Figure 1: Multidimensional scaling (MDS) based on Bray-Curtis distance comparing the extraction protocols used to analyse the Ash metabolome.

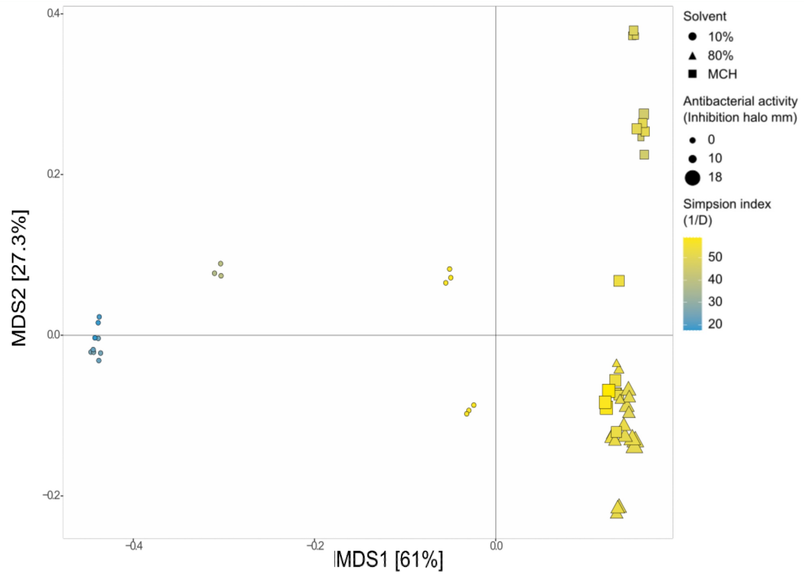


Figure 2: Multidimensional scaling (MDS) based on Bray-curtis distance comparing the extraction protocols used to analyse the Ash metabolome.

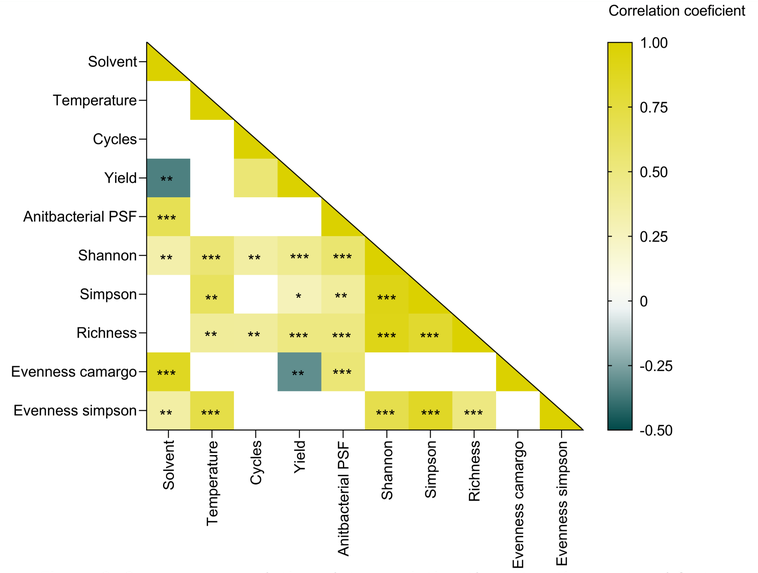


*Figure 3: Spearman correlation plot associating the extraction protocol factors and the variables. (*) as follows: ***p < 0.001, **p < 0.01, *p < 0.0 response*

Results

- Results show that clustering according to the factors tested (*Figure 1*)
- The solvents is having an significant effect on the diversity of chemical extracted, due to the varying polarity properties (*Figure 1&2*)
- Diversity is also dependant on the temperature (*Figure 2*), In the case of 10% MeOH, increasing temperature increase diversity.
- Solvent correlated with (inversely) Yield and Anti-bacterial activity, Temperature& cycles is significantly correlated with richness (*Figure 3*)

Conclusion and recommendation

- Do different protocols influence the overall metabolites extracted? Yes
- What is the best protocol for extracting ash metabolites?
  - High temperature 50°C
  - Non-polar (for anti-bacterial activity)- MCH
  - Polar (for yield) – 10%
- Overall- 80% Methanol at 50°C with 3 cycles

References:

1) Janse, J.D. (1981). European Journal of Forest Pathology, 11: 306-315. https://doi.org/10.1111/j.1439-0329.1981.tb00100.x
2) M. Urpi-Sarda, et al, Curr. Cardiol. (2015)
3) Amanda et al., RSC Adv., 2014, 4, 26325. DOI: 10.1039/c4ra02731k
4) Kellogg et al., 2017, JPBA, https://doi.org/10.1016/j.jpba.2017.07.027

Method illustrations are made in Biorender.