



Detection of Phylloxera Infestation in Grape Vines using NMR Methods

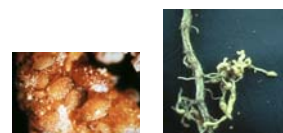
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- Phylloxera**
- gradually destroys the vine's root system, reducing capacity to take up water & nutrients
 - infested vineyards can lose 20% of production in the first year of infestation
 - vines generally become uneconomic within 3 to 10 years of infestation
 - ultimately requires replacement with vines grafted onto resistant rootstocks
 - replacement of infested vines with resistant rootstocks costs \$20,000-\$25,000 per hectare
 - only 15% of Australia's grape vines are currently planted on resistant rootstocks
 - a rigorous quarantine system is used to prevent spread of the pest
 - detected by excavation and investigation of the fibrous root system for galls and insects



Can Phylloxera infestation cause detectable metabolic changes in grape leaves?

We are investigating the existence of chemical fingerprints of Phylloxera in extracts taken from grapevine leaves and their possible use as a method of early detection of Phylloxera infestation.

Method

- Collection Site - Mount Helens vineyard, Upton, Vic. Cab. Sauvignon - Block 20 (uninfested), Block 21 (infested)
- Collection Dates - November, 2003; January, 2004; March, 2004. In November and January, one large leaf and three small leaves were collected from each of 15 vines. In March only large leaves were collected.
- Preparation - leaves were dried for 48 hours at 45° then extracted with dichloromethane (25ml) for 48 hours.
- ¹H NMR spectra measured at 750MHz in CDCl₃ solution (9 mg / 700 μ), analysed by wavelet transform or PCA.



Preliminary Results

November 2003 Collection - large leaves - Wavelet transform (4th level of resolution) shows significant differences in energies between infested (n = 8) and uninfested (n = 7) vines at locations 2, 3 and 4 (see fig 1 - E4.2, E4.3 and E4.4).
Mean E4.4 values (log scale) are: infested - 19.1 +/- 0.2; uninfested - 18.3 +/- 0.2
small leaves - no significant differences were found in wavelet energies.

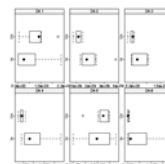


Figure 1
Wavelet transform
Box plot of energies of fourth
level of resolution



Principal component analysis

Figure 2
Scores Plot - PC1 v PC2
All collections - Large leaves only
Blue - November uninfested
Purple - November infested
Green - January uninfested
Red - January infested
Orange - March uninfested
Light - March infested

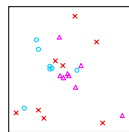


Figure 3
Scores Plot - PC1 v PC2
November collection - Large leaves only
Blue - uninfested
Purple - infested
Red - uninfested (leaves extracted
2 months after first batch)

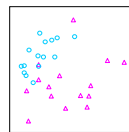


Figure 4
Scores Plot - PC1 v PC2
January collection - Large leaves only
Blue - uninfested
Purple - infested

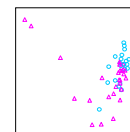
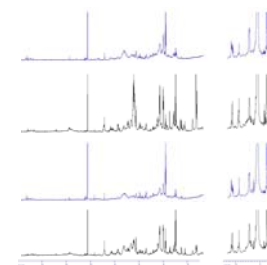


Figure 5
Scores Plot - PC1 v PC2
March collection
Blue - uninfested
Purple - infested

Preliminary Conclusions

- There are significant differences in the spectra of DCM extracts of leaves from infested and uninfested vines collected at the same stage of the growing season.
- All extractions of a collection should be conducted at the same time as dried grape leaves apparently undergo changes upon storage at room temperature (see fig 3).
- Collections made in January appear to give the best distinction (coinciding with the period of peak Phylloxera population in the season) but significant differences are apparent as early as November (soon after emergence).



Typical ¹H NMR spectra
November Collection
Lower two spectra -
uninfested vine
(Block 20 Row 17 Vine 10)
Upper two spectra -
infested vine
(Block 21 Row 21 Vine 10)
Blue - small leaves
Black - large leaves
vertical scale expanded five
times for left hand region
(10 - 2.5 ppm)

Further Questions

- Are these differences specific to Phylloxera or a more general response to stress?
We have conducted glass house trials, incorporating Phylloxera, water and nutrient stress to answer this - results not yet available.
- What chemical changes are responsible for these differences and can we find a convenient analytical method to detect them?

The CRC for Viticulture is a joint venture between the following core participants, working with a wide range of supporting participants.

