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Summary report for research undertaken at the "Environmental Analytical Laboratories, Charles Sturt University, Wagga Wagga"

This project aims to determine how allelochemicals found in Australian native mistletoe interact with the surrounding flora and fauna and how this may impact mistletoe distribution. Increased CO_2 levels have been shown to significantly increase the concentration of allelochemicals such as tannins, phenolics, and cyanogenic glycosides in leaves and a decrease in leaf nitrogen, resulting in reduced nutritional value (e.g. Gleadow et al. 1998 Plant, Cell Environ. **21**, 12; 2009 Plant Biol, **11**, 76). It is intended that the results from this study will provide a baseline for mistletoe allelochemicals which can be used to quantify the effects of increased CO_2 and aridity on mistletoe toxicity and hence distribution.

In order to obtain sufficient field replication, this study has focussed on the particular mistletoe/host combination *Amyema quandang* (grey mistletoe) and *Acacia dealbata* (silver wattle). *Acacia dealbata* is found in abundance within several locations in South Eastern Australia including regenerating woodland and urban environments. Whilst it can host several species of mistletoe it has an affinity with *Amyema quandang* and both species can usually be located at sites which contain sufficient replication. In order to determine the nature of the infection and whether a natural 'immunity' to mistletoe infection exists, *Acacia dealbata* trees which were in the vicinity of infected trees but remained uninfected were also sampled. Watson, (pers. comm.) indicated that trees which remained uninfected in high infection areas were not as a result of lack of seed dispersal but a lack of seedling establishment. A possible explanation for this is a difference in allelochemicals leading to 'immunity' within some individuals.

An initial screening for a wide range of common allelochemicals which could have been present in *Amyema quandang* and *Acacia dealbata* was conducted at Monash University. During this process, initial positive results have led this project to focus on hydrolysable tannins and phenolic compounds. Both these compounds are carbon based and therefore toxicity is likely to increase with predicted changes in atmospheric CO_2 (Koricheva et. al. 1998 Okios, **83**, 212).

The purpose of my visit to the "Environmental Analytical Laboratories (EAL)" to work with Dr. Min An has been to establish a successful protocol for the extraction and analysis of *Amyema* and *Acacia* compounds using one of his LC MS instruments. The instrument was optimised for tannin and phenolics analysis. This first trip has seen the successful development of novel protocols for the extraction and analysis of both *Amyema* and *Acacia* foliar samples. Results suggest that *Amyema quandang* has a unique profile containing high concentrations of several compounds not seen in *Acacia dealbata*. The overall concentration of tannins and phenolics found in *Amyema quandang* can be related back to the initial site they were sampled from. With the exception of a few peaks, the profile of *Acacia dealbata* was very different to *Amyema quandang*. There are also a few, but inconsistent differences between the profiles and concentrations of infected and non-infected *Acacia dealbata*. Statistical analysis will be needed to establish if those differences are significant. Overall concentrations of tannin and phenolic compounds varied between mistletoe and wattle samples with all mistletoe samples containing much higher concentrations. These results are also consistent with the initial whole plant analysis conducted at Monash University. In addition, there were differences in the overall concentration of 'Total Condensed Tannins' and 'Total Phenolics' found in samples from different sites suggest that environmental factors are contributing to these results. Carbon and Nitrogen analysis of foliar tissue also implies an influence of site on defence profiles. The differences in concentration are much more pronounced in the parasite supporting the idea of mistletoe as a keystone species.

The results obtained during this visit to the "EAL" are a first look into the allelochemical profiles of a species of Australian mistletoe and its host. These preliminary results suggest that both these species studied have the produce potentially toxic allelochemicals, and that the concentrations in which these are produced is dependent on environmental factors. A second visit is planned for January 2012 to complete the analysis for all remaining samples. The allelochemicals found in these species are carbon based. Research into the effect of atmospheric carbon on carbon based compounds has shown that such compounds are influenced by atmospheric CO_2 and with predicted increases in CO_2 become increasingly toxic to consumers.