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Author:	T.V. Steinrucken, A.B. Bissett, J.R. Powell, R.D. Van Klinken, A.K.H Raghavendra
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Dieback occurrence in a pastoral weed is associated with changes in endophyte community composition

T.V. Steinrucken^{1*}, A.B. Bissett², J.R. Powell¹, R.D. Van Klinken³, A.K.H Raghavendra³

¹ Hawkesbury Institute for the Environment, University of Western Sydney, Penrith, Australia, 2751.

² CSIRO Agriculture Flagship, Black Mountain, Canberra, Australia, 2601,

³ CSIRO Biosecurity Flagship, Ecosciences Precinct, Dutton Park, Australia, 4102

Corresponding author: tracey.steinrucken@csiro.au

The thorny tree, *Parkinsonia aculeata*, was introduced to Australia from meso-America in the 1800s has since become a Weed of National Significance (Van Klinken et al, 2009). It forms dense, impenetrable thickets that reduce carrying capacity, pasture availability, access to water, and prevents mustering. Unexplained dieback of some populations has been observed and is thought to be due to infection by pathogens within affected plants (Diplock et al, 2006). Determining the cause of dieback in parkinsonia could lead to more efficient management strategies to reduce the economic, social and environmental impact of this weed. However, there are hundreds of fungal, bacterial and archaeal microorganisms within plants that may have beneficial, symbiotic or pathogenic roles. In order to get a complete picture of their presence or absence, we use a molecular fingerprinting technique called terminal restriction fragment length polymorphism (T-RFLP) to provide an overview of the total microbial community structure and determine how this relates to dieback in parkinsonia.

We sampled stems from two locally-occurring native tree species (one within the same family as parkinsonia), and roots, stems and stem tips from healthy and dieback-affected parkinsonia populations in Charters Towers, QLD. We extracted total DNA from the surface-sterilized samples and ran three nested-PCR reactions using fluorescently-labelled, taxon-specific primers for archaea, bacteria and fungi with reaction conditions as in Singh and Thomas (2006). This was followed by a mono-digestion using HindIII and analysis on a sequencer to produce unique T-RFs (terminal restriction fragments) representative of each species within the archaeal, fungal and bacterial communities. Statistical analysis of the data compared microbial community composition by host specificity, between healthy and dieback-affected parkinsonia and across above- and below-ground plant parts.

We found that only archaeal community composition is host-dependent but that this might be due to the geographical origin of the host rather than inter-species relatedness since T-RFs in parkinsonia and *Bauhinia cunninghamii* – both Fabaceae – show more variation than those in the two unrelated native species (Table 1). Bacterial community composition showed no significant correlation to either plant part or dieback presence, but both factors correspond to a shift in archaeal and fungal endophyte communities (Table 2) in parkinsonia tips and roots.

Table 1. Pairwise permutational MANOVA of archaeal endophyte community data by host species. Data summarised from stem samples only.

Pairwise comparison	t	P(perm)
<i>P. aculeata</i> , <i>B. cunninghamii</i>	3.5612	0.0001
<i>P. aculeata</i> , <i>Melaleuca sp.</i>	3.1786	0.0005
<i>B. cunninghamii</i> , <i>Melaleuca sp.</i>	1.5138	0.0112

Table 2. Pairwise permutational MANOVA of archaeal and fungal endophyte T-RFs by disease status within *P. aculeata* plant parts

	Archaea		Fungi	
	t	P(perm)	t	P(perm)
Tip	2.1696	0.0001	1.7748	0.0001
Stem	1.2535	0.0616	1.5601	0.0036
Root	2.1696	0.0001	1.491	0.0016

This study shows that invasive tree species may host unique archaeal communities compared to native tree species, and that archaeal and fungal communities in parkinsonia tips and roots change in the presence of dieback. In order to draw inference from these correlations, future research will focus on the identification of individual endophytes so that potential pathogens can be tested via glasshouse and field-based inoculation experiments.

Diplock, N., et al. 2006. Proceedings of the 15th AWC: 585-587

Singh, B.K. & Thomas, N. 2006. *Nature protocols*, **1**: 2428-33

van Klinken, R. D., et al. 2009. *Plant Protection Quarterly*, **24**: 100-117