## Using genetic tools to diagnose dieback in Parkinsonia aculeata

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**Summary** Dieback in invasive and agricultural plants is a common phenomenon attributed to a range of abiotic and biotic factors. In this project we are investigating the link between the introduction and establishment of a non-native plant to a new range and the subsequent dying-back of the invasive population. We are focusing on possible pathogen-related causes for dieback, especially considering the following three scenarios:

- 1. re-association with pathogens from the nonnative's native range,
- new association with pathogens in the introduced range, and
- 3. association with cosmopolitan pathogens.

A diverse range of pathogens – in particular fungi – are often associated with many of the observed dieback events, but determining causality has often been surprisingly difficult. This may in part be due to interactions with other biotic and abiotic stressors. In this paper we hypothesise that the composition of the endophytic microbial community (including fungi, bacteria and archaea) of *Parkinsonia aculeata* L. (parkinsonia), an invasive non-native plant in the introduced range, might contribute to the occurrence of dieback within populations of this invasive plant.

Parkinsonia, a leguminous shrub from the Americas, is one of Australia's most economically important Weeds of National Significance (WoNS), covering millions of hectares across Queensland, Western Australia and the Northern Territory, and impacting on pasture availability, water access and biodiversity (van Klinken and Heard 2012). Dieback in parkinsonia has been observed for a number of years, and in some cases kills entire populations (Diplock *et al.* 2006).

Previously investigations have focused on the potential for fungal endophytes to be the cause of dieback in parkinsonia, and found that a number of these endophytes are pathogenic to this species *in vitro* (Diplock *et al.* 2006). However, the possibility that a

complex of pathogens are responsible for dieback has not been considered. Neither has the involvement of other microorganisms such as bacteria in the etiology of the disease. The objective of this study therefore, is to compare the microbial community composition of various plant parts of dieback-affected and healthy parkinsonia plants, and native trees to determine if there is a relationship with the occurrence of dieback in parkinsonia. A relationship between the microbial endophyte communities and the dieback phenomenon in parkinsonia, will aid in the development of a model for predicting the occurrence and severity of dieback in parkinsonia populations.

Samples were taken from stems, roots, stem tips and seeds of dieback-affected parkinsonia, healthy parkinsonia and local native trees near Charters Towers, Queensland in May 2013. After surface sterilisation, DNA was extracted from internal tissue samples and endophytic bacterial, fungal and archaeal communities were PCR amplified according to Singh and Thomas (2006). The analysis of this data is currently underway using Primer-E (Ramette 2009) to determine if microbial community composition in various parts of parkinsonia or native trees contributes to the occurrence of dieback, or results in a trend that can be related to dieback occurrence.

While the dying-back of weeds is ecologically and economically beneficial, further research into finding the causes of this phenomenon for each case of dieback, could lead to the development of more effective and more targeted control methods for these unwanted species.

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## REFERENCES

- Diplock, N., Galea V., van Klinken, R.D. and Wearing, A. (2006). A preliminary investigation of dieback on *Parkinsonia aculeata*. Proceedings of the 15th Australian Weeds Conference, eds C. Preston, J.H. Watts and N.D. Crossman, pp. 585-7. (Weed Management Society of South Australia Inc.).
- Ramette, A. (2009). Quantitative community fingerprinting methods for estimating the abundance of operational taxonomic units in natural microbial
- communities. *Applied and Environmental Microbiology* 75: 8, 2495-2505.
- Singh, B.K. and Thomas, N. (2006). Multiplex-terminal restriction fragment length polymorphism. *Nature protocols* 1: 5, 2428-33.
- van Klinken, R.D. and Heard, T.A. (2012). *Parkinsonia aculeata* L. parkinsonia. *In* 'Biological control of weeds in Australia', eds M. Julien, R. McFadyen and J. Cullen, pp. 437-46. (CSIRO Publishing, Collingwood).