

## Evaluation of fungal isolates for potential use as mycoherbicides for seed bank reduction of *Parkinsonia aculeata*

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**Summary** *Parkinsonia (Parkinsonia aculeata* L., Fabaceae) is an exotic, leguminous woody weed that threatens much of rangeland Australia. A dieback disorder has been widely observed on parkinsonia plants in Australia. This study focuses on screening isolates, collected from affected trees in the field, against germinating parkinsonia seedlings. Preliminary results show that some of these isolates are able to cause death within 12 days of inoculation, indicating potential for development of a pathogen-based mycoherbicide to reduce recruitment.

**Keywords** *Parkinsonia*, dieback, seedlings, isolate screening, biocontrol agent.

### INTRODUCTION

*Parkinsonia* is a weed of national significance that impacts on the environment and the pastoral industry in rangeland Australia (Deveze 2004). A dieback disorder which causes high mortality of parkinsonia plants has been widely observed in Australia for at least several decades. As part of a study of this phenomenon, approximately 200 fungal accessions have been isolated from dieback-affected trees across Australia (N. Diplock unpublished data). To date, three different genera from this isolate bank have been shown to be capable of inducing significant stem lesions on parkinsonia trees after inoculation (N. Diplock unpublished data), thereby supporting the possibility of developing mycoherbicides based on endemic fungal pathogens.

*Parkinsonia* seed is protected by a tough coat and is tolerant of most environmental conditions. Dormancy can be broken by hot, wet conditions (van Klinken *et al.* 2006) and fire (Deveze 2004). Seed germination in northern Australia occurs during the wet season (generally December to March) (Deveze 2004). Ecological research has shown that seedlings are particularly sensitive to fire, drought or inundation (van Klinken unpublished data).

In this paper, 84 fungal isolates were carefully selected from an isolate bank maintained at the University of Queensland (Diplock *et al.* 2006) to represent different collection sites and morphological characteristics to ensure the widest range of possibilities. These isolates were screened for pathogenicity against

germinating parkinsonia seedlings using a specific bioassay. The aim of this study was to determine whether any of these isolates were lethal to seedlings, and therefore might have potential as mycoherbicides.

### MATERIALS AND METHODS

**Preparation of inoculum** Millet seed was rinsed twice in distilled water, soaked for 24 hours, and rinsed for a third time before being transferred into plastic McCartney tubes (11.60 g prepared millet seed per tube), and autoclaved twice within 24 hours (N. Diplock unpublished method).

Fungal isolates were initially divided into 16 locations according to the GPS coordinates of the collection sites (N. Diplock unpublished data), and replicates were removed from the list. Sub-culturing was carried out from master culture plates using half-strength PDA (potato dextrose agar), according to the selection list. The fungi were incubated at 25°C.

In cases where bacterial contamination was present, full-strength PDA with antibiotics was used. This medium contained Penicillin (Sigma<sup>®</sup>, Penicillin G sodium salt) 300 ppm and Streptomycin (Sigma<sup>®</sup>, Streptomycin sulfate salt) 200 ppm.

The tubes containing autoclaved millet were inoculated with a 10 × 10 mm piece of actively growing culture and incubated at 25°C until fully colonised. The tubes were then placed unsealed in the laminar flow cabinet and allowed to partially dehydrate and were then stored at 4°C.

**Seed germination** Intact seeds (collected from the Caerphilly Station near Charters Towers, QLD) were washed with sterile water, bleached for five minutes in 2% NaOCl, and washed again with sterile water, then dried in the laminar flow cabinet before being clipped at the end nearest to the embryo groove, with a disinfected nail clipper (J. McKenzie personal communication). Clipped seeds were germinated in surface-sterilised Petri dishes on moist sterile filter paper in darkness at 25°C. Seeds were deemed ready for transplanting once the radicle extended over half the length of the seed (generally after two days of incubation).

**Test arena** Sixteen sterile transparent plastic boxes (Décor®, 1.75 L, with lids and a 6 mm diameter ventilation hole on each side), were used as incubation compartments. Each box contained a single treatment. Transparent plastic tubes (25 mm internal diameter × 77 mm internal height) with a single 4 mm diameter hole in the base, were grouped 16 per box. Each was filled to 20 mm height with sterile Vermiculite and supported on a plastic mesh base to provide free drainage.

**Isolate screening** One replicate was initially conducted on all 84 isolates to determine their pathogenicity. A further two replicates were subsequently conducted on the eight most aggressive isolates. Twelve randomly selected isolates were tested at a time, together with two positive and two negative control sets.

The screening was conducted by adding three grains of colonised millet (fungal inoculum) into each prepared (Vermiculite) tube. One day later, a single germinated seed was transplanted into each tube and covered with 10 mm of autoclaved Vermiculite. Boxes were then sealed and randomly arranged in a constant temperature room (30°C, 14:8 hours light: dark) and incubated for 12 days with watering every fourth day. The positive control was prepared by adding three grains of millet colonised by an aggressive fungal isolate (NT039), and the negative control used autoclaved millet (no pathogen).

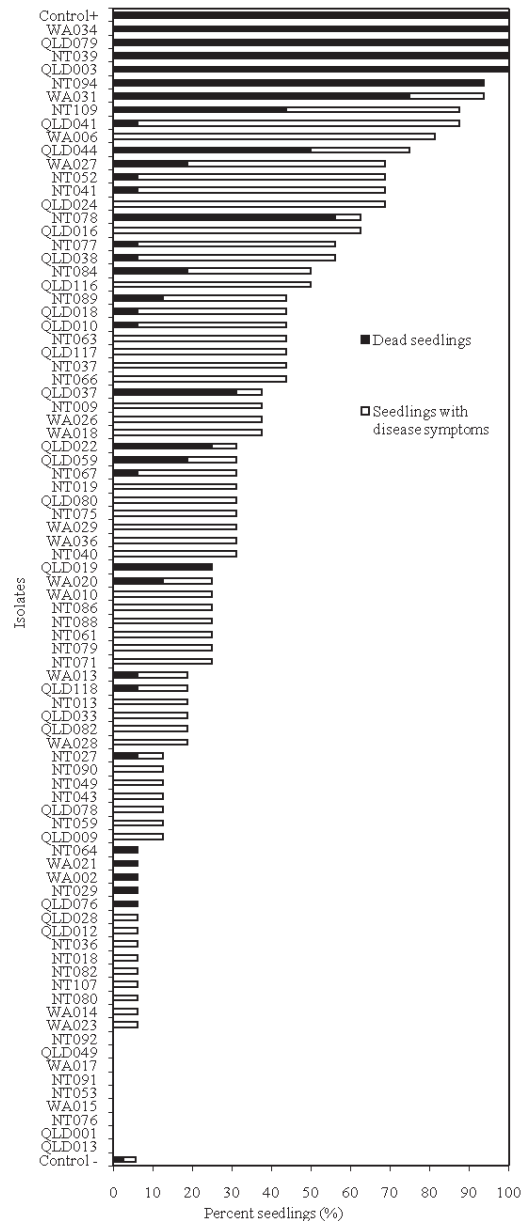
**Assessment of disease** Seedling health was categorised every four days as either dead, showing disease symptoms or healthy.

## RESULTS AND DISCUSSION

The positive and negative controls caused very high and very low mortality respectively (Figure 1). Considerable variation in pathogenicity exists among the 84 isolates individually tested, indicating that the bioassay developed for this research is of appropriate sensitivity to provide separation among isolates. Furthermore, these results indicate that across its Australian range, parkinsonia appears to host a selection of organisms which display varying degrees of pathogenicity.

Most isolates caused disease symptoms rather than death to seedlings within the 12 day observation period. However, it is possible that some of these isolates may have caused higher seedling mortality if the trial had been run for longer. The most lethal isolates caused seedling death so rapidly that many seedlings did not even emerge above the growing media.

The high mortality rates caused by the eight most lethal isolates in the initial screening were supported by replication (Table 1). Of those, five isolates resulted



**Figure 1.** The 84 isolates tested ordered by decreasing aggressiveness, together with the pooled positive controls and pooled negative controls.

in similar mortality rates to the positive control (Table 1), and will therefore be considered further for their potential as mycoherbicides.

**Table 1.** List of the eight most lethal isolates (Figure 1) compared to positive and negative controls.

Isolate	ID	Origin	Mean % mortality (95% c.i.)
Control+	<i>Lasiodiplodia</i> sp. 1	McArthur River Mine, NT	99.6 <sup>a</sup> (97.2 – 99.9)
WA034	Unidentified	Kununurra, WA	100.0 <sup>a</sup>
NT039	<i>Lasiodiplodia</i> sp. 1	McArthur River Mine, NT	100.0 <sup>a</sup>
QLD079	<i>Lasiodiplodia</i> sp. 2	Fletchervale, QLD	97.9 <sup>a</sup> (86.6 – 99.7)
QLD003	<i>Fusicoccum</i>	Fletchervale, QLD	97.9 <sup>a</sup> (86.6 – 99.7)
NT094	Unidentified	Newcastle Waters, NT	97.9 <sup>a</sup> (86.6 – 99.7)
WA031	Unidentified	Halls Creek, WA	79.1 <sup>b</sup> (65.4 – 88.4)
QLD044	Unidentified	Hughenden, QLD	66.6 <sup>b</sup> (52.3 – 78.4)
NT109	Unidentified	Tennant Creek, NT	50.0 <sup>b</sup> (36.2 – 63.7)
Control–			2.3 <sup>c</sup> (1.0 – 5.1)

<sup>1</sup>Different letters signify significant differences (P <0.05).

Three of the five have been identified as either *Lasiodiplodia* or *Fusicoccum* (Table 1). Species in these genera are known to cause wilt, canker, and dieback on a wide host range in the tropics and subtropics (Punithalingam 1979, J. Cunnington personal communication). However, they have not yet been recorded from field-collected parkinsonia seeds and seedlings.

Our results show that pathogens can cause very high seedling mortality under laboratory conditions. Priorities now are to determine whether they cause similar impacts under natural conditions, whether impacts can be increased through management actions, and to assess the potential of these isolates as mycoherbicides.

#### ACKNOWLEDGMENTS

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