A preliminary investigation of dieback on *Parkinsonia aculeata*

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Summary Parkinsonia (*Parkinsonia aculeata* L. Fabaceae) is a serious woody weed in rangeland Australia that has been observed showing signs of dieback across northern Australia. This disorder has not previously been studied in depth, but is known to kill individual plants and, sometimes, whole stands of parkinsonia. A field trial was conducted to test whether a selection of fungal organisms isolated from affected plants are causative agents of dieback and, ultimately, their potential use as biological control agents. This study showed that the two isolates tested were effective in causing disease in parkinsonia when applied as a stem inoculation.

Keywords Parkinsonia, dieback, biological control, fungal agents.

INTRODUCTION

Parkinsonia has been identified as one of Australia's top 20 weeds, threatening to invade much of mainland Australia (Deveze 2004). Currently, control of parkinsonia is mostly conducted by chemical and mechanical means (Deveze 2004) but its application in many areas is restricted by constraints such as environmental concerns and costs. Three biological control agents have been released with limited success (Julien and Griffiths 1998, van Klinken 2004, van Klinken 2005).

However, high unexplained mortality within parkinsonia populations have been commonly observed across Australia for many decades (van Klinken, unpublished data). This phenomenon is commonly referred to as parkinsonia dieback and results in substantial control in some areas. Manipulation of this phenomenon may offer a new control tool.

In some observed cases, plants suffering from dieback show symptoms starting from the tip of the branch, which begins to die back, with phyllodes and pinnae drying up and remaining attached to the plant. A distinct dark, necrotic region on the stem appears to move down the plant as the disease develops. When stems of affected branches are cut open, vascular staining is often evident. Subsequent isolations from affected plants collected in Qld, NT and WA have yielded a selection of fungal isolates.

In this paper, preliminary results of a field trial conducted to test whether two isolates extracted from dieback-affected trees are likely to be the causative agents of dieback are presented. Trials have been carried out in various locations to determine which of these isolates may be responsible for the dieback phenomenon. This paper will discuss the preliminary results of a trial carried out near Charters Towers, Qld.

MATERIALS AND METHODS

Isolate acquisition Stems collected from Fletchervale, QLD (approximately 200 km from trial field site) showing symptoms of disease, were cut into pieces approximately 1 cm in length. Care was taken not to use material from the ends of the stem segments to avoid contamination by surface micro-organisms. These segments were surface sterilised using 4% NaOCl for three minutes, rinsed in sterile water for three minutes, followed by a second rinse in sterile water for one minute. The surface sterile segments were then placed on half strength potato dextrose agar (½PDA) plates using sterile forceps. These plates were then placed in an incubator at 25°C and observed daily.

When fungal hyphae was observed growing from the stem segments, sub cultures were made onto new ½PDA plates. Plates were observed daily, and examined under the microscope periodically. When the culture covered most of the plate, the plate was placed under UV light for 24 hours to encourage sporulation.

Preparation of inoculum Two different isolates were used, QLD001 and QLD003 from a single plant collected at Fletchervale, as well as a control treatment of autoclaved millet. Millet seed (50 g) was placed in 250 mL Erlenmeyer flasks, and soaked with deionised water for 24 hours. These were capped with cotton wool and aluminium foil. Excess water was then poured off and the millet was autoclaved twice at 121°C for 25 minutes, 24 hours apart. After the millet had cooled, approximately 5 mL of sterile water was added to each flask. Portions of fungal cultures grown on ½PDA were used to inoculate the millet flasks,

which were then placed in an incubator at 25°C. After the first three days, flasks were shaken weekly. Full colonisation of the millet was achieved following three weeks of incubation.

Approximately 2 g of millet inoculum was rolled into 'cigarettes' using a cigarette rolling machine (Cougar brand) and cigarette papers (Tally-ho brand). The rolling machine and the working area were carefully disinfected with methylated spirits between cultures.

Tree selection and inoculation The trial site was located on Taemas Station, approximately 120 km south of Charters Towers, QLD (21°05'09.2"S, 146°25'59.6"E). Dieback had not previously been observed at this location. Healthy, adult trees (with stem circumferences between 10 and 14 cm) were selected in six blocks of ten and individually tagged. Trees were inoculated on 24th June 2005. In each block five randomly selected trees were root-inoculated and five were stem-inoculated (Table 1). Two of these blocks were inoculated with QLD001, two with QLD003 and two with controls.

Tree assessments All plants were rated six months after inoculation for plant vigour, leaflet cover and stem lesion presence (and length). Vigour was rated by estimating the plant's rachis cover (recorded on a scale of 0–10, (with 0 = dead, no foliage, and 10 being fully foliated). Leaflet cover was measured as a percentage of the maximum number possible and stem lesion length was measured in cm. Root wounds were not assessed.

Statistical analysis All statistical analyses were conducted in Minitab Version 14. A one way unstacked ANOVA (P = 0.05) was used to compare individual treatments to each other.

RESULTS

Root inoculation of the two isolates had no significant effect (P = 0.05) on lesion size, leaflet cover or plant vigour when compared with controls, (data not shown). In contrast, stem inoculation of the two isolates resulted in significantly larger stem lesions (Figure 1) and a small but significant reduction in leaflet cover (Figure 2) and vigour (Figure 3).

Isolates QLD003 and QLD001 appeared to be pathogenic to parkinsonia and resulted in large stem cankers on stem inoculated plants as compared with the control, which showed very little signs of plant damage (Figure 4). Control plants showed a general trend of healing over completely, or scarring and healing of the tissue around the wound (Figure 4a). In fungal-inoculated plants, the wound remained open, and very little scar tissue was visible (Figure 4b). Lesions were generally located on tissue surrounding

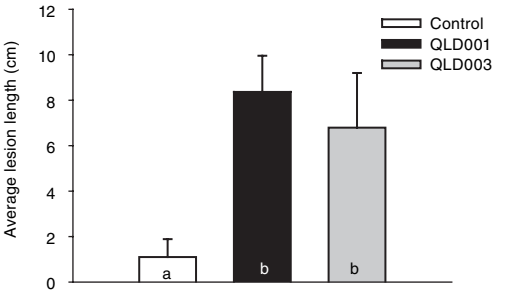

Figure 1. Average lesion length of stem wounded plants, six months after inoculation. Different letters indicate a significant difference between treatments.

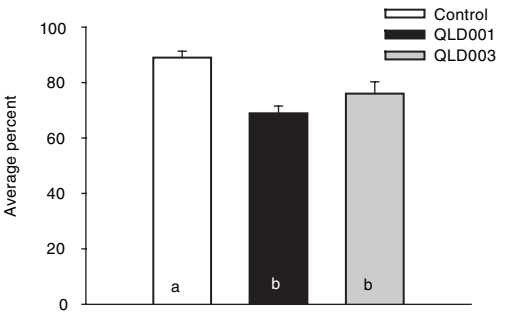

Figure 2. Average percentage of attached leaflets on stem wounded plants, six months after inoculation. Different letters indicate a significant difference between treatments.

Table 1. Inoculation methods.

	Wound method	Inoculation method
Root inoculation	Soil removed from base of plant to reveal one root ≈1 mm in diameter and 5 cm long. Outer surface of root scraped	One 'cigarette' was placed along side root, approx. 5 mL of sterile water squirted onto 'cigarette', soil hole covered over
Stem inoculation	Stem drilled at approx. 35–70° angle with 6 mm drill bit, 10 cm above soil level	One 'cigarette' placed in the drilled hole. Approx. 5 mL of sterile water squirted into hole, wound covered with plastic (gaffer) tape

the wound; however, on some plants the lesions were located on the opposite side of the stem. Wounds in the fungal-inoculated treatments had a definite fungal/bacterial aroma to them. Many of the wounds were surrounded by spongy (decaying) wood. This was a clear indication of pathogenic activity as these wounds failed to heal following inoculation as they did in the control treatment.

DISCUSSION

Stem inoculation of the both isolates resulted in large lesions and a significant decrease in plant health after only six months. Both isolates used were originally extracted from parkinsonia plants that were suffering from dieback symptoms. This is therefore the first concrete evidence that parkinsonia dieback is caused by one or more pathogens. Isolations from these plants will however, be taken in the future to confirm the pathogens applied to the plants were the actual cause of the disease observed in this trial.

It is unknown how this disease transmits itself naturally between individual trees and stands of parkinsonia. However, the dose rate applied in this trial is almost certainly much higher than would be expected naturally. This is seen in the disease symptom development, with lesions in this trial being more dramatic that what is observed naturally.

In stem-inoculated plants, inoculation with either isolate resulted in disease development and increased plant stress compared to the control treatment. Root-inoculated plants did not show significant development of disease symptoms in any of the treatments. This may indicate that the inoculum is most effective when enclosed in a stem wound, where conditions for infection are more favourable. The availability of moisture required for infection may be more likely within the stem wound hole than in the soil making this a more effective technique.

Overall vigour was significantly reduced in stem-wounded plants inoculated with QLD001 and QLD003. This suggests that disease was beginning to manifest itself to a point were it was significantly reducing plant health. Leaflet drop gives an indication of stress symptoms of parkinsonia (Hocking 1993) and this was apparent with the stem inoculated (fungal) plants.

These initial results are promising and indicate that the isolates QLD001 and QLD003 are potentially pathogenic to parkinsonia plants. Further assessments of this trial are planned.

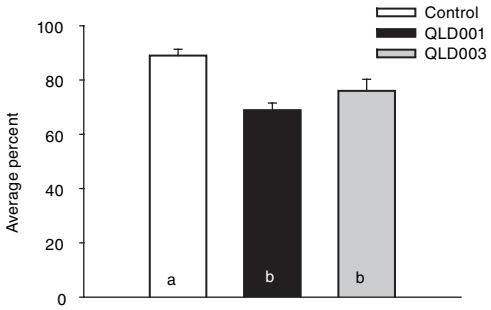

Figure 3. Average vigour of stem wounded plants, six months after inoculation. Different letters indicate a significant difference between treatments.

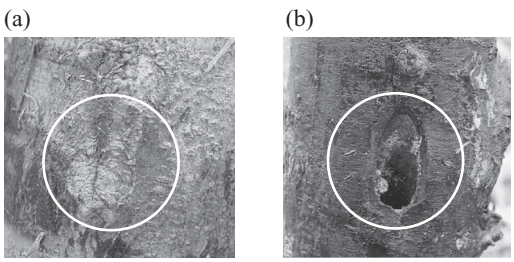

Figure 4. (a) Healed stem wound on control treatment, (b) stem wound on plant inoculated with QLD003.

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