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Article

Use of stem implanted bioherbicide capsules to manage an infestation of *Parkinsonia aculeata* in northern Australia

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Abstract: An infestation of parkinsonia (*Parkinsonia aculeata*) located on Alexandria Station, Northern Territory, Australia was successfully treated with a bioherbicide using stem implanted capsules. The bioherbicide containing three endemic endophytic fungi (*Lasiodiplodia pseudotheobromae*, *Macrophomina phaseolina* and *Neoscytalidium novaehollandiae*) is the first Australian registered woody weed bioherbicide.

The product was effectively administered to the plant stems using a mechanical device, resulting in subsequent development of a dieback event, which, after a period of establishment, has moved through the adjacent untreated plant population resulting in significant decline in infestation vigour and reduced recruitment. This is the first report of large-scale management of parkinsonia by this method.

Keywords: stem implanted capsule; bioherbicide; parkinsonia; woody weed; dieback, mechanical delivery

1. Introduction

Parkinsonia (*Parkinsonia aculeata* L.) also known as Mexican palo, Jerusalem thorn, blue palo verde, horse bean tree, sessaban and Barbados flower fence [1] is a thorny shrub that can grow into a small tree with a natural range from the southern USA through to Argentina [2]. Parkinsonia is thought to have been introduced in Australia in the 1860s [3] quickly spreading through its utility as an ornamental shade tree and by 1906, was considered as a weed [4]. Parkinsonia populations are greatest in northern Australia (Queensland, Northern Territory and Western Australia), with only a few scattered infestations reported in New South Wales and even fewer found in South Australia. It is estimated to be present over 3.3 million ha of Australia [3] and has been classified as a Weed of National Significance (WoNS) in recognition of it being a major weed threat. Parkinsonia is considered a major weed threat in Australia and is on the Weeds of National Significance (WoNS) register. This classification identifies parkinsonia for implementation of strategic action plans (at various jurisdictional levels) as a priority species for management programs and targeted research as it produces impenetrable thickets which impact on grazing systems [5] and natural riparian zones.

Dieback of parkinsonia is a documented phenomenon leading to plant mortality and sometimes entire populations are affected [5]. The first detailed examination of parkinsonia dieback across northern Australia [6] resulted in the identification of 41 fungal species from 13 families. Among these, eight species from the family Botryosphaeriaceae were found to be common across all

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five climatic regions sampled in dieback affected parkinsonia. A seven-year study of a naturally occurring dieback event near Hughenden, Queensland [6,7] demonstrated the linear movement of this disorder through a parkinsonia population killing established plants and preventing recruitment of juveniles. An inoculation methodology progressively developed in that study [6] proved successful in establishing dieback within healthy individuals using French white millet (grain) colonised with certain species selected from the fungal bank developed in that project. Subsequent research developed an advanced process [8] in which colonised millet was formulated in either gelatin or hypromellose pharmaceutical capsules and subsequently utilised by other members of this research group [9,10,11]. This technology based on Australian patent # AU 2009201231 B2 was further developed into a mechanized delivery system [12] which was used in this research.

Glasshouse studies by another research group [13] using their own isolates of endophytic fungi made from both healthy and dieback affected parkinsonia populations near Charters Towers (north Queensland) and a benchmark isolate (*Lasiodiplodia pseudotheobromae* – NT039) selected from this laboratory [6, 14], failed to produce dieback symptoms or systemic infection in juvenile parkinsonia plants under three water stress regimes.

Although parkinsonia dieback affected plants may be associated with a variety of endophytic fungi, possibly as latent pathogens, the authors [13] suggested that the syndrome itself may require an external environmental trigger to initiate disease which implies that inoculation alone may not be sufficient to induce disease in the field.

The current study aimed to evaluate the effectiveness of a stem implanted bioherbicide for the control of a vigorous population of parkinsonia growing naturally in a rangeland situation. This study also reports the first large scale use of a mechanized delivery system to enable rapid and effective inoculation of parkinsonia.

2. Materials and Methods

The location for this study was an isolated site identified as Corporal Dam (S18^{O}59’15” E137^{O}39’27”) on Alexandria Station, a large cattle property (16,116 km^2) in the Barkly Tablelands region of the Northern Territory Australia (Figure 1). The vegetation type is best described as a Mitchell grass (*Astrebla*) tussock grassland, [15] a tropical savannah bioregion characterised by flat or rolling country dominated by C4 grasses and mostly lacking in trees. More specifically, it can be described as a Tropical Arid Grassland (MVG19-0) under the Australian major vegetation classification system [16]. The infestation of parkinsonia was located around a dam and its immediate catchment area. This is a typical habitat for this riparian weed as parkinsonia is generally associated with waterways and sites where cattle travel and congregate.

The study site consisted of two areas of reasonably dense and healthy parkinsonia. The smaller (1.9 ha) north infestation was adjacent to the dam (0.6 ha) with a population estimated to be 4,000 plants. The larger (11.2 ha) south infestation was similarly densely covered with a population estimated at 24,000 trees (Figure 2).

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Figure 1. Location of Alexandria Station in the Northern Territory and location of the Northern Territory (shaded red inset) within Australia [17].

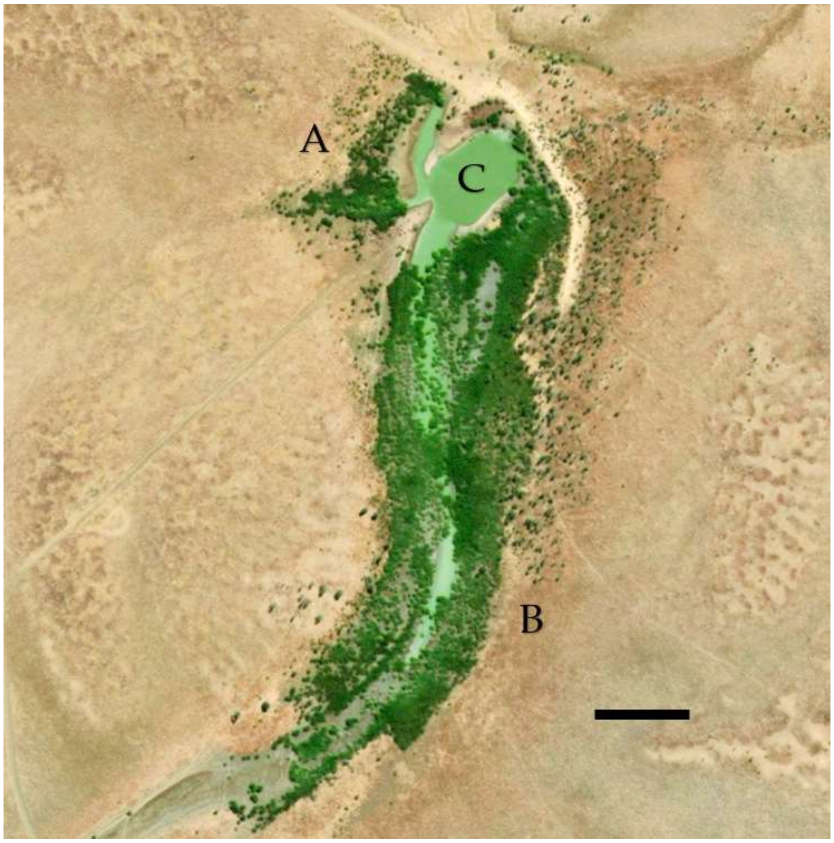

Figure 2. Satellite image of Corporal Dam study site with north infestation (A) south infestation (B) and dam (C). Vegetation almost entirely consisting of *Parkinsonia aculeata*. Scale bar = 100m. Image [18] dated 2017.

The study commenced in August 2016. The north infestation was chosen for high density inoculation with the bioherbicide Di-Bak Parkinsonia®

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(BioHerbicides Australia Pty Ltd, Queensland, Australia) which at the time was in development, and as of December 2018 is registered through the Australian Pesticides and Veterinary Medicines Authority (APVMA; https://apvma.gov.au/). Di-Bak Parkinsonia is a formulation of three endophytic fungi from the phylum Ascomycetes (order Botryosphaeriales, family *Botryosphaeriaceae*), *Lasiodiplodia pseudotheobromae* A.J.L. Phillips, A. Alves & Crous (strain NT039), *Macrophomina phaseolina* (Tassi) Goid. (strain NT094) and *Neoscytalidium novaehollandiae* Pavlic, T.I.Burgess & M.J.Wingf. (strain QLD003) that have been cultured separately on autoclaved white millet (*Panicum miliaceum* L.), dehydrated, combined, and placed into size 0 hypromellose pharmaceutical grade capsules (filled weight approximately 0.32g). The south infestation was selected for limited treatment with the same bioherbicide to examine the potential for lateral movement of dieback from treated areas to adjacent plants. At commencement of the study, the overall health of plants at the site was observed to be excellent. Close observation through the subsequent treatment process (1,785 trees), and extensive examination (more than 5,000 trees) confirmed the site to be free of dieback (Figures 3a & 3b).

(a)

(b)

Figure 3. (a) Densely crowded healthy parkinsonia plants in north infestation; (**b**) Free standing healthy parkinsonia in south infestation at trial commencement.

Inoculation of trees was achieved with the use of a prototype applicator device operated manually and powered by a cordless drill (Figure 4a). The applicator utilizes an 8mm diameter drill bit to create a 25mm deep hole in the tree stem (at a height of 10-30cm above soil level) while located firmly by sharp points pushed into the stem While the operator maintains forward pressure on the applicator against the tree, the action of withdrawing the drill fully primes the device with a single capsule (21.6x7.6mm) and a sealing plug (15x7.5mm) which are in tandem within each of the 15 chambers in the magazine (Figure 4b). The capsule and plug are then rapidly inserted into the fresh drill hole with a light plunging motion achieved by pushing the cordless drill forward using the drill bit as a ram. The capsule is lightly compressed within the hole with the plug end clearly visible on the stem surface (Figure 4c). As the capsules contain bioherbicidal fungi, a single dose only is delivered to a plant, irrespective of its size resulting in the development of a stem lesion within months of treatment (Figure 4d).

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(a) (b)

(c) (d)

Figure 4. (a) Implanting a bioherbicide capsule into the lower stem of parkinsonia using a mechanical applicator; (b) Loading the magazine with bioherbicide capsules and sealing plugs; (c) A treated stem with the sealing plug partially protruding from the treatment hole; (d) A parkinsonia stem six months after treatment showing a visible stem lesion in proximity to the treatment site.

North infestation

Treatment of the densely populated north infestation was conducted according to the following principles: Where trees were in relative proximity (less than 0.5m apart), the plant with the largest stem was treated before moving away at least 1m to repeat the process. Where plants were more widely spaced or completely isolated, each tree with a stem thick enough to implant with a capsule (at least 40mm diameter) was treated. This approach allowed the operators to move effectively throughout the infestation ensuring good coverage of treatment taking into consideration plant density and distribution. Measurement of the distribution of treatments was achieved by recording a GPS waypoint (Garmin GPSmap 62s) at the point where a full magazine (15 doses) was initiated, and at every

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subsequent magazine change. On completion of treatment, the GPS unit was used to map the external perimeter of the whole treated area and calculate its area (1.9 ha). Of the estimated 4,000 trees, 1,080 (72 magazines) were implanted with bioherbicide capsules (Figure 5).

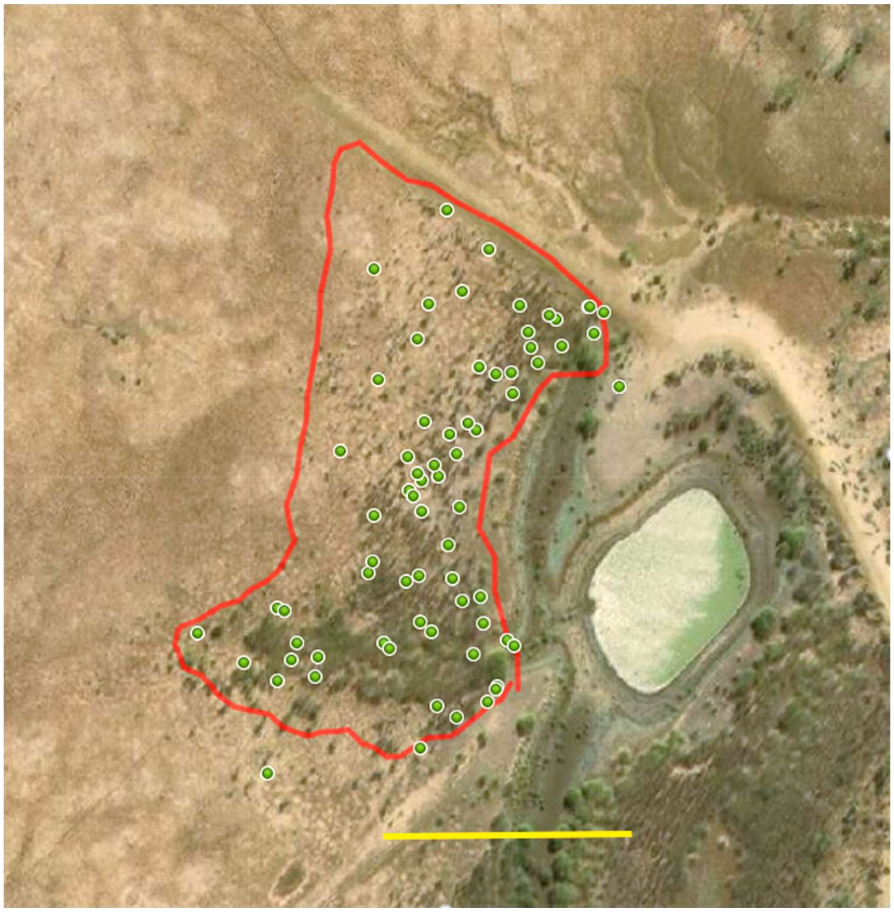

Figure 5. Outline (in red) of north infestation boundary as tracked by GPS surrounding the treatment area (1.9ha) green markers indicating waypoints at which magazines of 15 doses were initiated. Scale bar = 100m. Background imagery [19] accessed April 2021.

South infestation

Treatment of the south infestation (Figure 6) was limited to the southern most tip and restricted to the western side of the creek line which terminated at the dam to the north. Given that the population in this site was of lower density, and trees were generally of a larger size than in the north infestation, the inoculation strategy employed here was to treat every tree in this limited area with a single implanted bioherbicide capsule. The intention was to establish a seat of dieback with the potential to move in the direction of water flow [6,7]. The infestation boundary was determined by GPS tracking as descibed above (yielding an area of 11.2 ha). In total, 705 trees were implanted requiring the use of 47 magazines with waypoints captured as described above. The total parkinsonia population was estimated at 24,000 trees. Work rate ranged between 101 and 145 trees per hour for the equipment operators across both the north and south infestations.

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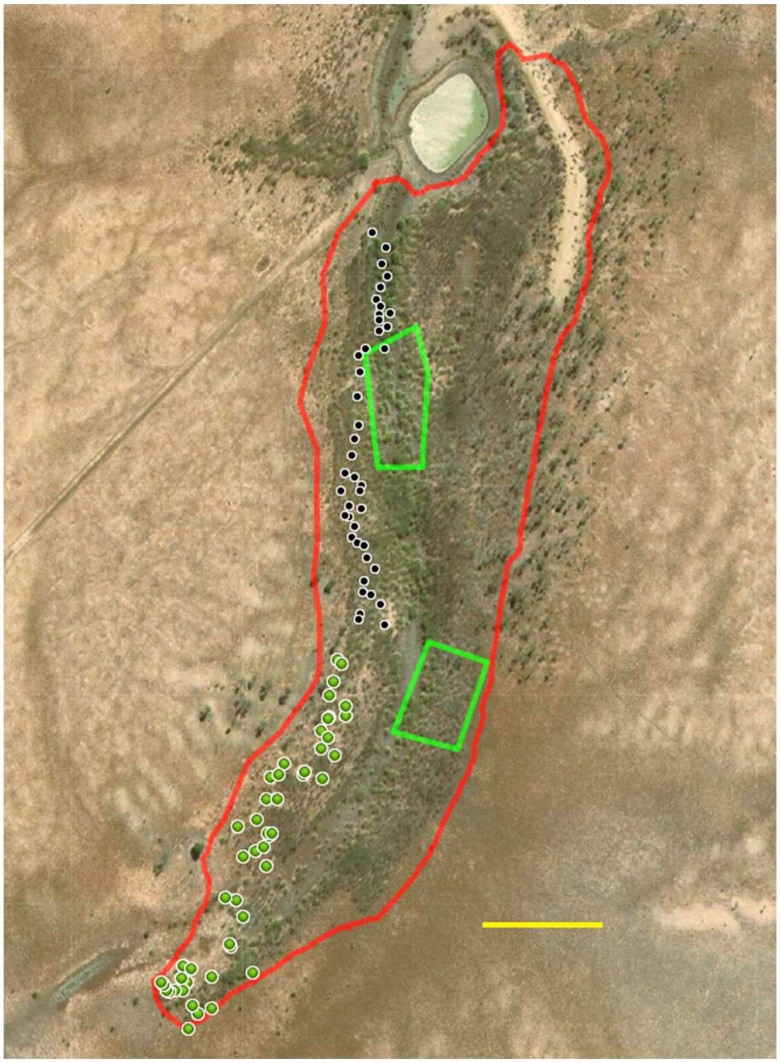

Figure 6. Outline (in red) of south infestation boundary as tracked by GPS (11.2ha), green circle markers indicating waypoints at which magazines of 15 doses were initiated. Light green boundaries indicate reference (control) sectors. Black circle markers indicate assessment points for linear transect. Scale bar = 100m. Background imagery [19] accessed April 2021.

Reference areas

Two sectors approximating rectangles were selected within the eastern side of the creek line within the south infestation as untreated reference (control) areas (Figure 6) with critical waypoints captured by GPS. The size of these sectors was 4,250 and 3,730 m² respectively containing more than 800 trees each.

Rating system and assessment dates

Parkinsonia tree health was evaluated using a modification of a visual scoring system [6] as outlined in Table 1. Each tree was carefully assessed to determine health by estimation of the percentage of the tree structure (stem and branches) that were considered alive. Leaves are not considered a valuable determinant of plant condition, as this species readily sheds them under conditions of water stress. Healthy parkinsonia have green stems and branches which transition to yellow (chlorotic), then to red (severely chlorotic), followed by brown and black (necrotic) pigmentation as dieback develops. As parkinsonia trees grow, the loss of some functional material (generally lower branch tips) is observed in normal (non-dieback affected) plants, hence the rating class 5 is valuable in differentiating highly healthy trees from those showing mild symptoms of dieback (class 4).

Table 1. Modified tree health scoring system to evaluate condition of parkinsonia under field conditions.

Numeric Rating	Descripton of Tree Condition
5	>95% of tree remaining green and alive
4	71 – 95% of tree remaining green and alive
3	51 – 70% of tree remaining green and alive
2	31 – 50% of tree remaining green and alive
1	1 - 30% of tree remaining green and alive
0	Dead tree

Assessments were carried out on the north infestation at trial establishment (week 0) and at 45, 64, 165 and 221 weeks after treatment (WAT). At 0 WAT, 600 trees were assessed by rating three groups of 200 (which were further divided into clusters of 20) to establish baseline condition of the experimental site. This was achieved by walking through each assessment area (the north infestation – Figure 5, and the two reference sectors – Figure 6) in a random pattern using GPS to ensure that repeat assessments of individual trees did not occur. Navigation in the more densely populated sections was made difficult, but not impossible, due to the thorny trees and dense nature of the infestation.

The north infestation was assessed for changes in health score at each subsequent time point (45, 64, 165 & 221 WAT). For the first two assessments, this was limited only to 200 treated trees (10 clusters of 20 each) which could be identified by either the presence of the inoculation plug, or where the plug had been lost, by the presence of a drill hole. At 165 WAT, 960 (treated) trees were easily identified for assessment, whereas, by 221 WAT, widespread tree death, lodging and the coating of tree stems with mud from a previous flooding event made identification of treated trees by plug presence or dill holes problematic. For this last assessment, 1200 randomly selected trees (60 clusters of 20 each) across the whole treatment zone were scored representing approximately 30% of all

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trees in this study area. The untreated reference sectors (Figure 6) were assessed at 45 & 64 WAT by selecting 100 trees (5 X 20) from each of the two sectors, and then increasing this to 200 trees (10 x 20) per sector at 165 & 221 WAT.

Assessment of the south infestation was conducted at the last two assessment events only (165 & 221 WAT) by which time there was significant presence of dieback. As the trees in this study area were large and mostly free-standing with almost 100% treated (705 in total), assessment was conducted only on those trees where an inoculation site could be identified equating to 700 trees and 600 trees at 165 and 221 WAT respectively.

South infestation transect

At 221 WAT, general observations revealed that a significant swath of dieback affected plants on the eastern flank of the south infestation in what was previously observed to be a healthy zone between the treated zone (consisting of 705 treated trees) and the dam to the north (Figure 6). A sampling assessment approximating a linear transect was conducted from the northern (dam) end towards the previously treated (and dieback affected) southern zone. A true linear transect was impracticable due to restriction in navigating through the dense and clumped population of parkinsonia. The method employed was to record a GPS waypoint, and then assess (using the previously described method) 10 parkinsonia trees that could be seen in a circle from that position before walking south a minimum of 5 metres then repeating the process. In total, 40 assessments were made (400 trees) over a linear distance of 290m.

Weather data

Rainfall data collected by a weather station at the Gallipoli outstation 28 km southeast of the trial site was provided by the North Australian Pastoral Company (NAPCo, Brisbane, Queensland). Temperature data was accessed from the nearest location (Camooweal Township, S19^{O}55′12″ E138^{O}7′12″) 114 km SE of Corporal Dam [20].

Statistical methods

Data analysis was performed with RStudio (V 4.1.0) using linear mixed models with post-hoc testing and comparison of means was conducted by Tukey's HSD test at 0.01 confidence level. This approach considered the variations in sample size which increased as the trial progressed and the categorical nature of the rating scales.

3. Results

3.1. Weather data

Data for the site (Figure 7a) indicates that in the 12 months following establishment of the trial (August 2016 – August 2017), rainfall was within the expected range, however the following 12 months were unusually dry with the failure of the 2017-2018 wet season, and similarly less than average rainfall in the 2018-2019 season with a return to expected levels for the 2019-2020 season. Temperature readings from Camooweal over the period of the trial (Figure 7b) indicate that monthly mean minima ranged between 8.5 and 26.9^{o}C while the monthly mean maxima ranged between 25.7 and 42.0^{o}C.

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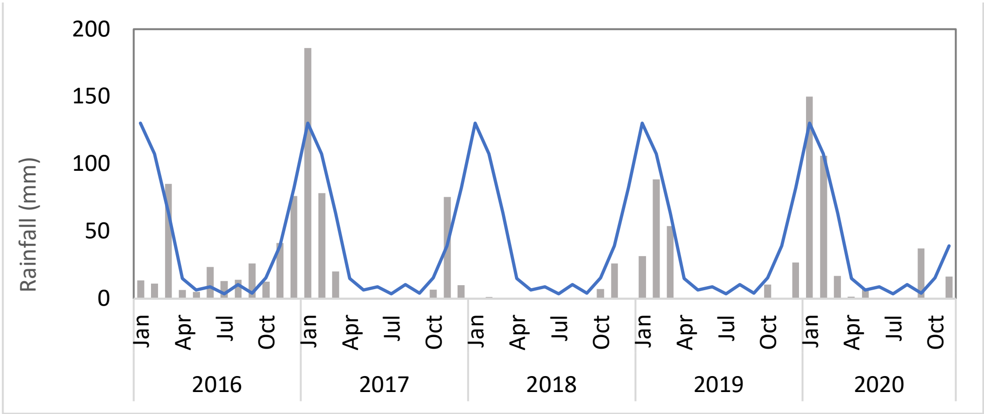
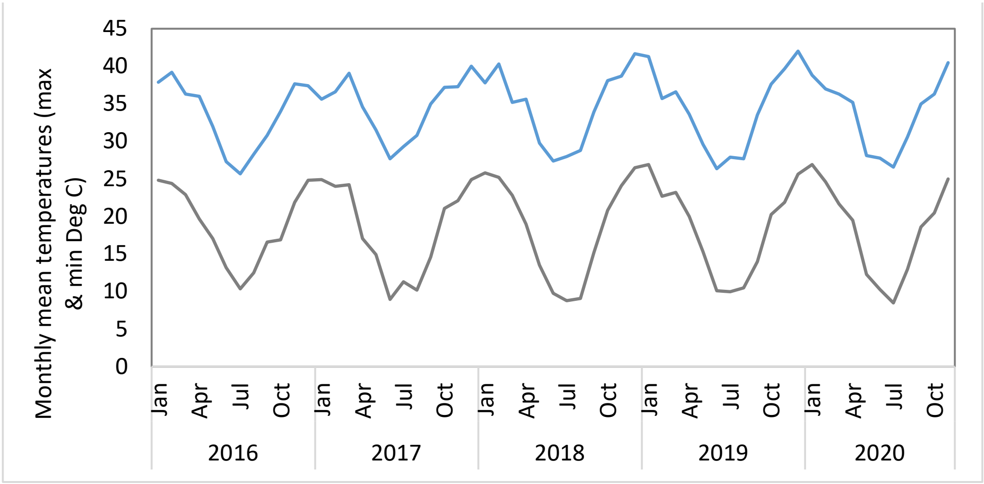


(a)



(b)

Figure 7. (a) Monthly rainfall totals (bars) recorded at Gallipoli outstation from January 2016 to October 2019. The line is indicative of the ten year (2000 – 2019) monthly rainfall means. Data source, NAPCo. (b) Monthly mean maximum and mean minimum temperatures from Camooweal weather station [20].

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3.2. Dieback development in the North Infestation

Development of dieback in the north infestation was incremental and significantly greater ($p < 0.01$) than that observed in the untreated control areas as evidenced by the steadily falling health scores for all assessment dates after treatment (Figure 8). Health had fallen to a rating of 3.82 by 64 WAT, then to 1.18 and 0.35 by 165 and 221 WAT. There was a comparatively smaller reduction in health in untreated plots (falling from 5.0 to 4.34 and 4.36 by 165 and 221 weeks respectively. Although these reductions in health were significant ($p < 0.01$), they are minimal and were expected as a consequence of an aging plant population.

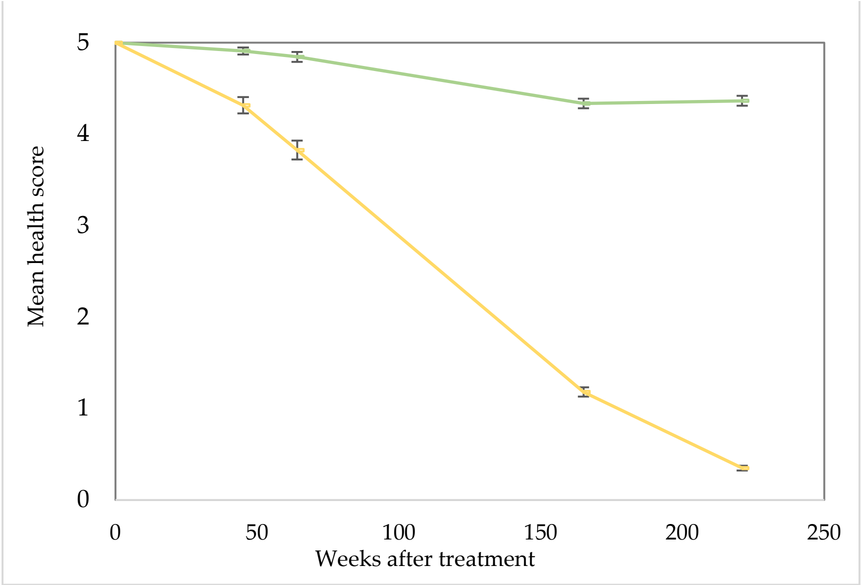

Figure 8. North infestation parkinsonia overall health score over trial duration (0, 45, 64, 165 & 221 WAT). Treated plants (gold), untreated control plants (green). Error bars represent ± standard error of means.

Mortality assessment (Figure 9) indicates that falling health scores rapidly translated into dead parkinsonia plants. At 45, 64, 165 and 221 WAT mortality averaged 6.0, 9.0, 55.3 and 81.1% with each increment significantly ($p < 0.01$) greater than the previous. Visually, plant mortality was evident (Figure 10) with treated trees mostly dead, in some cases having fallen over or if remaining standing, often shedding their bark. The lesser increment in mortality among the untreated control steadily (and significantly; $p < 0.01$) increased from 1.0, 1.5, 7.8% and stabilized at 7.9% over the same period.

Clearer visualisation of the process of dieback development through the duration of the trial across all experiments can be seen in the stacked bar representation (Figure 11). While there was gradual, but low background levels of dieback apparent in the untreated areas (control) which was capped at around 20%, disease progress in the treated sites showed clear acceleration indicating a movement from class 5 downwards resulting in high levels of mortality and almost negligible proportions of healthy plants remaining at 221 WAT.

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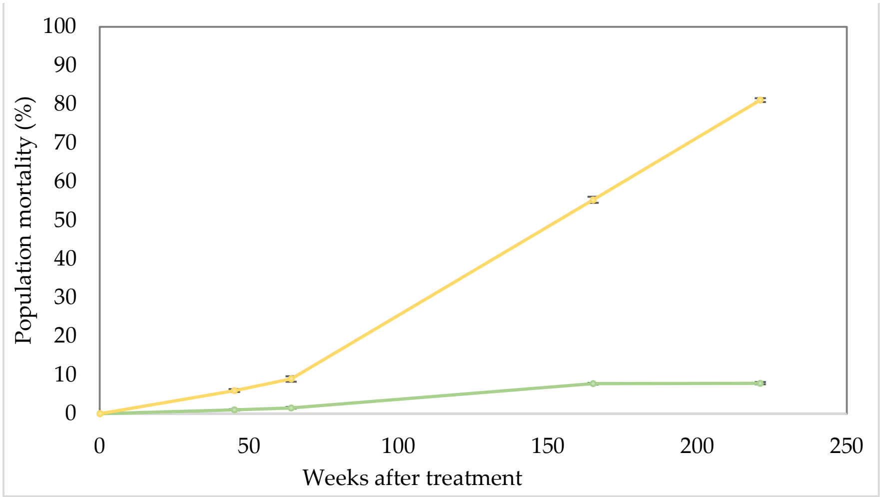
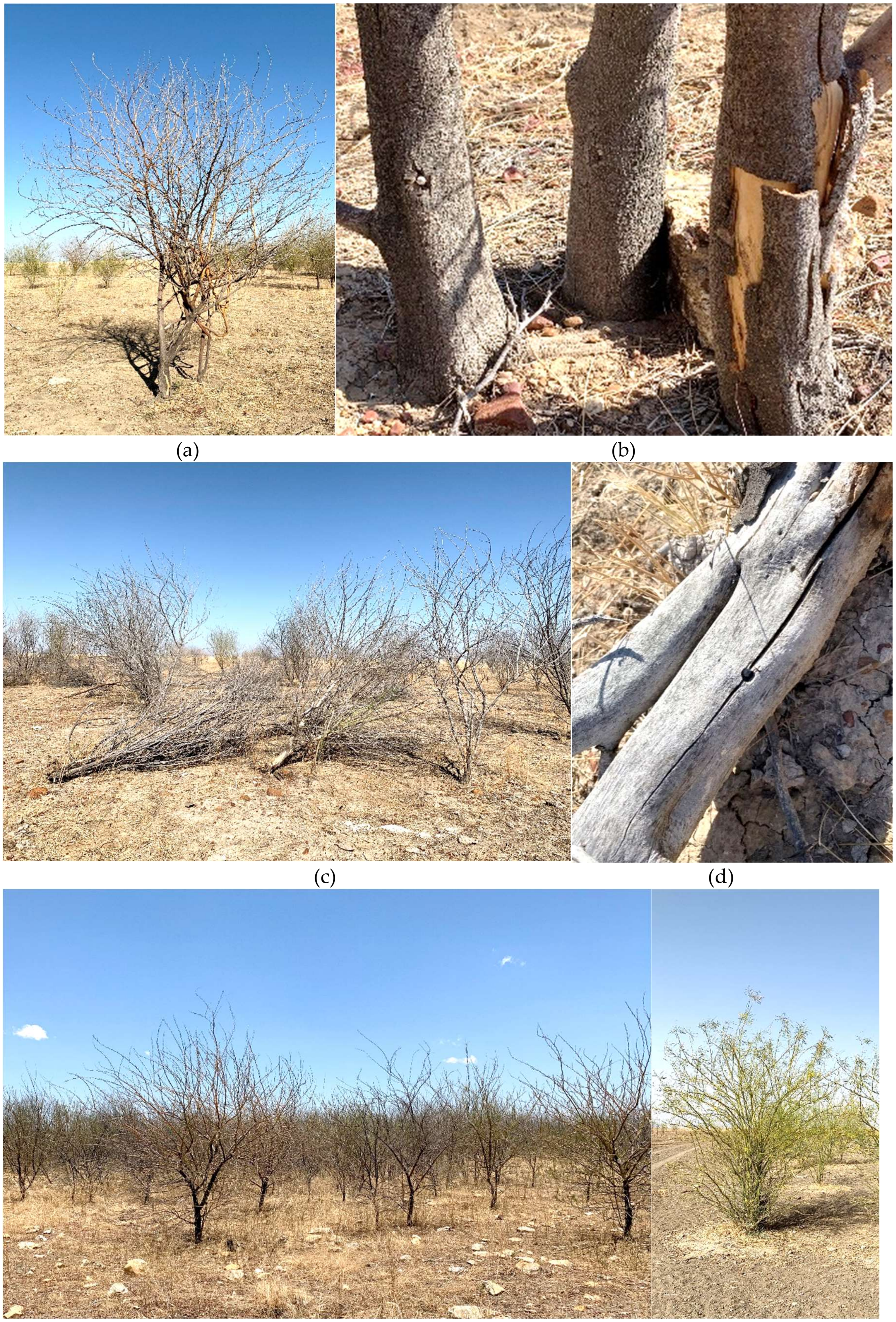


Figure 9. North infestation parkinsonia mortality over trial duration (0, 45, 64, 165 & 221 WAT). Treated plants (gold), untreated control plants (green). Error bars represent ± standard error of means.



(a) (b) (c) (d) (e) (f)

Figure 10. Result of parkinsonia treatment with implanted bioherbicide capsules at 221 weeks after treatment. (a) free standing dead parkinsonia tree, (b) three individually treated stems with bark shedding evident on right specimen, (c) fallen dead specimens in north infestation, (d) stem of fallen dead specimen with

inoculation hole evident, (e) effective and widespread mortality in treated are of south infestation, (f) live, healthy tree in untreated (control) area.

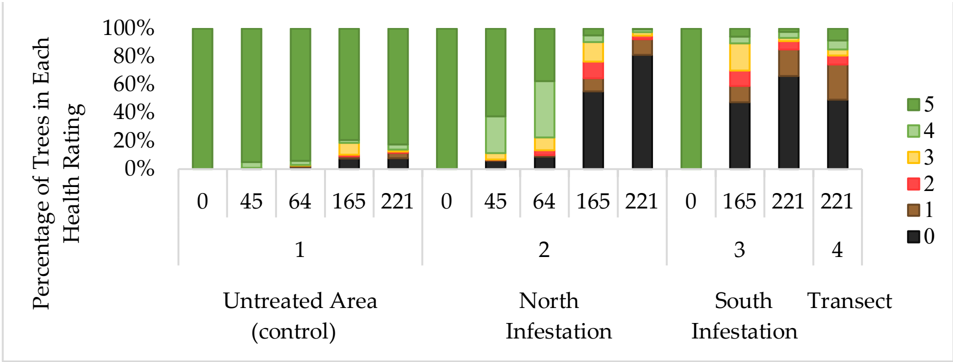


Figure 11. Progress of parkinsonia dieback represented as percentage distribution of each health rating class across control (1), north (2) and south infestations (3) over time (WAT) with comparison to transect (4) assessment. Rating 5 = highest health score (>95% alive); 4 (71 - 95% alive); 3 (51- 70% alive); 2 (31 - 50% alive); 1 (<30% alive); 0 (tree assessed as dead).

3.3. Dieback development in the South Infestation

Data collection for the south infestation was limited only to the last two assessment dates beyond the initial point of treatment (0 WAT). Decline in tree health among the inoculated trees was significantly greater ($p < 0.01$) than that displayed by the untreated (control) sections (Figures 11 & 12) falling to a health score of 1.40 and 0.67 at 165 and 221 WAT respectively in a trend similar to that seen in the north infestation (Figure 8).

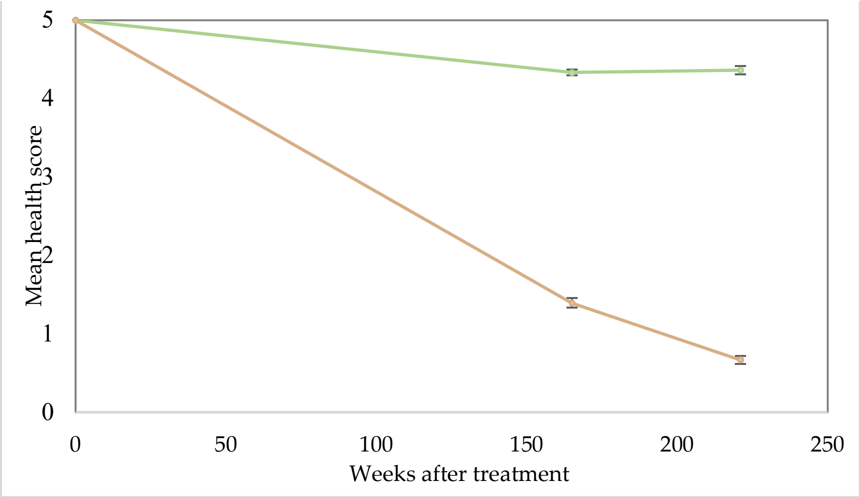


Figure 12. South infestation parkinsonia overall health score over trial duration (0, 165 & 221 WAT). Treated plants (gold), untreated control plants (green). Error bars represent ± standard error of means.

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Mortality assessment (Figure 13) of the south infection indicates a steady climb in plant death with 47.6 and 66.2 % mortality at 165 and 221 WAT respectively. Mortality rates among the untreated (control) plots were the same data as for the north infestation and significantly lower ($p < 0.01$) than for the treated section stabilizing (7.8 and 7.9 %) at the last two assessments.

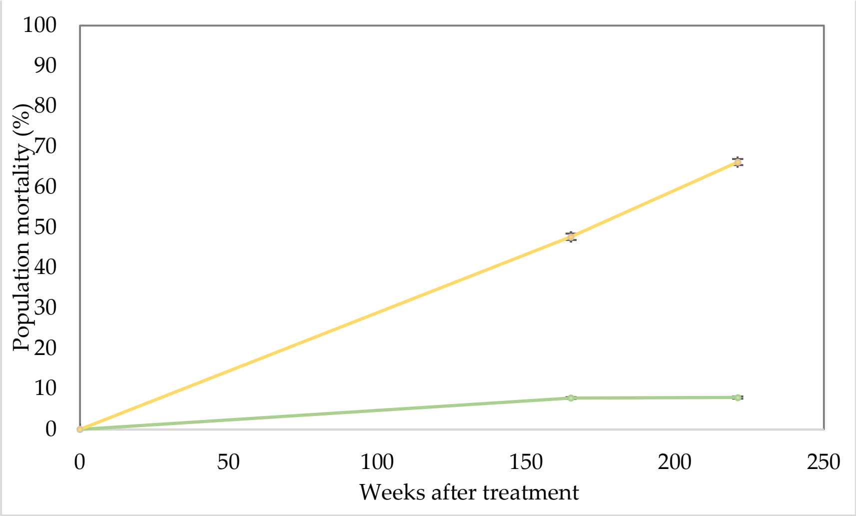

Figure 13. South infestation parkinsonia mortality over trial duration (0, 165 & 221 WAT). Treated plants (gold), untreated control plants (green). Error bars represent ± standard error of means.

3.4. South infestation transect

Overall plant health score across the 400 plants in the transect was low (Figure 11) at 1.19 compared to the untreated control at 4.36 ($p < 0.01$). Variability among assessment groups is illustrated by a heatmap diagram (Figure 14) showing the mean health score displayed by a colour chart. Among these data, only one cluster of 10 plants were rated in top health (class 5). Overall mortality was determined at 49.2% which was significantly greater ($p < 0.01$) than for the untreated control (7.8%).

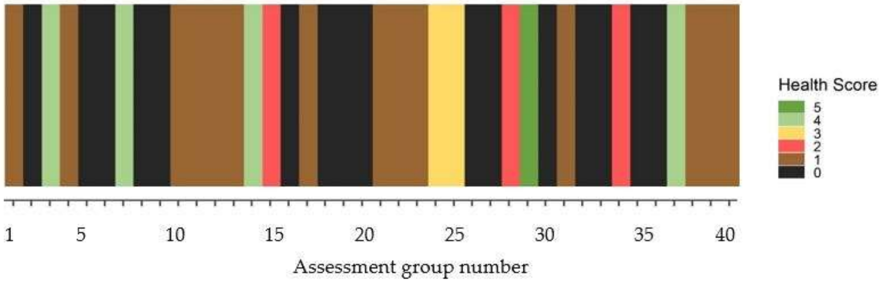

Figure 14. Heatmap of south infestation transect assessment points. Group 1 was located at the northern most point, Group 40 closest to the treated area to the south. Health score ratings from 5 (highest health level) to 0 (dead plant).

4. Discussion

Fungi in the family *Botryosphaeriaceae*, including those used in this study are known for their ability to act as saprophytes, endophytic colonizers, or as latent pathogens [21], for example, *M. phaseolina* (used in this study) is noted as one of the most destructive necrotrophic pathogens [22] due to its ability to produce a large repertoire of hydrolytic enzymes capable of degrading all major components of the plant cell wall and cuticle facilitating the infection process. Furthermore, this species, which is favored by higher temperatures (30-35^{o}C), produces an extensive array of enzymes and toxins that disarm host defense mechanisms causing cell death and tissue degradation [22] accelerating the process of colonisation, tissue necrosis and then plant death. More broadly, fungi in this family are well understood to initially colonise plants as endophytes, often infecting though natural openings, and then upon the onset of biotic and or abiotic stress, transition to a pathogenic strategy [21]. An examination of the climatic zones in which species were found to cause disease [23] assigned *M. phaseolina* to regions with a hot humid summer while *L. pseudotheobromae* (also used in this study) was assigned to regions with a warm humid summer. The study site is considered transitional between hot, dry summer, mild winter, and hot, humid summer. An examination of the *Botryosphaeriaceae* in Australia found that for species found in mango orchards in the Kimberley region, 90% could be isolated from adjacent native vegetation [23].

The results of this study clearly demonstrated that treatment of parkinsonia with stem implanted bioherbicide capsules resulted in successful infection, colonisation and movement of dieback resulting in plant mortality and apparent suppression of re-colonisation from the (soil) seed bank. The fungal isolates used in this study originated from locations [14] with hot humid summers [23], and within the same climatic zone as the study site, and therefore would be considered as well adapted to that temperature band.

Colonisation of treated trees was apparent at the first assessment point, 45 WAT, with stem lesions clearly apparent, a highly significant decrease in health score and a small, but significant increase in plant mortality. Health scores continued to fall and tree mortality continued to increase over the length of the trial while untreated controls remained relatively healthy over the same period of 221 weeks (4 years and 3 months). The overall outcome could only be described as an effective establishment (and continuing) dieback event in the 1.9 ha north infestation resulting in an overall 81% mortality rate. A full survey of the site on final assessment revealed no visible signs of seedling recruitment, supporting the evidence [6, 7, 14] that the isolates used in this study are effective in reducing seedling emergence by processes of pre and post emergent pathogenesis.

Treatment of the lower (up-stream) end of the south infestation similarly resulted in effective dieback development (98%), and substantial mortality (66%) by the conclusion of the trial. This treatment zone served as an inoculum source for those un-treated plants further north (downstream) with colonization evident for 290m towards the catchment point (Corporal Dam). This effective movement of the dieback front supported earlier observations [6,7] and clearly indicates that where parkinsonia has access to water movement in riparian locations, this method of treatment is effective in downstream dispersal of bioherbicide induced dieback. Furthermore, the ability of dieback to spread from plant to plant suggests that treatment levels (proportion of plants inoculated) may be significantly reduced, particularly where plants are densely populated. However, reducing treatment levels could arguably reduce the rate at which dieback develops at a treatment site and further modelling

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may help to establish a critical point to which treatment levels could be reduced to vastly increase the efficiency of the treatment process, thereby reducing the costs associated with managing this species.

A key factor in the success of the delivery mechanism is the rapid process of wounding and implantation and sealing of the inoculum capsule into the stem of the target plant. The access to moisture from xylem and phloem fluids and stem tissues, and the exclusion of an oxidizing and drying atmosphere to the wound tissues facilitates the absorption of moisture by the capsule contents resulting in the subsequent activation of the fungal agents and successful colonisation of the plant stem. As this is a process of inoculation by aggressive endophyte/pathogens which are known to be favored by wound entry [21], treatment success rates are understandably high. Furthermore, field observations have shown that the process is not dose dependent with large trees succumbing to inoculation with a single dose. The lightweight capsules, efficient and highly portable delivery system, and freedom from using liquid-based herbicides greatly improves the ability of an operator to rapidly treat a parkinsonia infestation compared to conventional methods. Furthermore, the species specificity of this approach eliminates the possibility of harming non-target species through environmental contamination.

A previous report on glasshouse experiments with parkinsonia failed to demonstrate the ability of endophytes isolated from diseased and healthy specimens to induce dieback symptoms [13] with the authors suggesting that inoculation alone may not be sufficient to cause disease in the field. The research reported here proves that inoculation under field conditions can successfully cause parkinsonia dieback in the field, and that it is capable of movement to untreated healthy plants both adjacent to and a substantial distance from the point of inoculation, supporting observations elsewhere [6,7] in naturally occurring study locations. Inducing dieback in glasshouse grown parkinsonia plants is problematic [13] with the absence of significant stress indicated as a potential key factor in this process. A major source of stress is environmental temperature, and the conditions (25°C) used by the authors [13] are significantly lower than those where fungi such as *M. phaseolina* [22] are most active (30-35°C). Monthly mean maximum temperatures observed nearby the study site for this research ranged between 25.7 and 42°C supporting the observation that under these much hotter conditions, inoculation of parkinsonia with a stem implanted bioherbicide capsule is an effective approach for initiating a dieback event leading to successful control of parkinsonia.

5. Conclusions

This is the first report of the use of capsule formulated bioherbicide delivered by a mechanized stem implantation device to successfully bring about large-scale control of *Parkinsonia aculeata*. The compact, robust, and convenient formulation of the bioherbicide as a capsule, the rapid delivery process, and the ability of the induced die-back which transmits through a plant population killing both adult trees and preventing recruitment from the seed bank creates a viable and environmentally appropriate alternative to conventional, labour-intensive physical and chemical control systems.

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References

1. Hawkins, J.A. *Parkinsonia aculeata* (Mexican palo-verde). In *Forestry Compendium*. (CABI Publishing, Oxon, UK), **2001**.
2. Polhill, R.M.; Vidal, J.E. Caesalpineae. In *Advances in legume systematics*, ed. R.M. Polhill, P.H. Raven, (Royal Botanic Gardens, Kew). **1981**; Volume 1, pp. 81-95.
3. van Klinken, R.D.; Campbell, S.D.; Heard, T.A.; McKenzie, J.; March, N. The biology of Australian weeds 54. *Parkinsonia aculeata* L. *Plant Protection Quarterly* **2009**, 24(3) pp. 100-117.
4. Bailey, F.M. *Weeds as suspected poison plants of Queensland;* H. Pole & Co.: Brisbane, Australia, **1906**.
5. March, N. Weeds of National Significance Parkinsonia (Parkinsonia aculeata L.) Strategic Plan 2012-17. Australian Government Department of Agriculture, Fisheries and Forestry, Canberra. Commonwealth of Australia. **2012** ISBN 978-1-921575-89-1
6. Diplock, N.D. Parkinsonia dieback: Investigations into its cause, ecology and potential for biological control. PhD, The University of Queensland, Queensland Australia, March **2016**.
7. Diplock N.D.; Galea V.J. Dynamics of dieback in a naturally occurring parkinsonia population. SciPlant 2017 – Science Protecting Plant Health (joint conference of the Australasian Plant Pathology Society and Plant Biosecurity CRC) September 26 – 28, **2017**. Brisbane, Australia. https://www.appsnet.org/publications/proceedings/2017%20abstracts.pdf
8. Galea, V.J.; Beilby, A. Parkinsonia Dieback Trials –Workshop and Field Manual. A cooperative research program to investigate the potential for managing parkinsonia using fungal biological control agents. ISBN: 978-1-864-99949-5 Savanna Solutions, Katherine, Northern Territory, Australia, **2009**.
9. Haque, A. Investigation of the fungi associated with dieback of prickly acacia (*Vachellia nilotica* subsp. *indica*) in Northern Australia. PhD, The University of Queensland, Queensland Australia, July **2015**.
10. Haque, A.; van Klinken, R.D.; Goulter, K.; Galea, V.J. Assessing the potential of fungi isolated from dieback-affected trees as biological control agents for prickly acacia (*Vachellia nilotica* subsp. *indica*). *BioControl* **2018**, 64(2), pp. 197-208. https://doi.org/10.1007/s10526-018-09919-9
11. Sacdalan, A.D. *Mimosa pigra* dieback in the Northern territory, Australia: Investigation into possible causes. PhD, The University of Queensland, Queensland Australia, December **2015**.
12. Galea, V.J.; Riikonen, P. Development of an effective delivery system for a woody weed bioherbicide. SciPlant 2017 – Science Protecting Plant Health (joint conference of the Australasian Plant Pathology Society and Plant Biosecurity CRC) September 26 – 28, **2017** Brisbane, Australia. https://www.appsnet.org/publications/proceedings/2017%20abstracts.pdf
13. Steinrucken, T.V.; Raghavendra, A.K.H.; Powell, J.R.; Bissett, A.; van Klinken, R.D. Triggering dieback in an invasive plant: endophyte diversity and pathogenicity. *Australasian Plant Pathol*. **2017**, 46: pp. 157-170. https://doi.org/10.1007/s13313-017-0472-5
14. Toh, R. Investigation of fungi pathogenic towards seedlings of *Parkinsonia aculeata* – their potential for use as mycoherbicides. M.Phil, The University of Queensland, Queensland Australia, November **2009**.
15. Australian Government (2015) Conservation Management Zones of Australia, Mitchell Grasslands. Department of the Environment (2015). https://www.environment.gov.au/system/files/resources/bfda11e1-1117-4d2c-9608-0b854f7ad21f/files/cmz-mitchell-grasslands.pdf (Accessed 12 Feb 2021).
16. Keith, D.A.; Pellow, B.J. Review of Australia's Major Vegetation classification and descriptions. Centre for Ecosystem Science, UNSW, Sydney, Australia **2015**. ISBN: 0-7334-3586-6
17. Wikipedia images https://en.wikipedia.org/wiki/Northern_Territory & https://en.wikipedia.org/wiki/File:Australia_location_map.svg (Accessed 12 July 2021).

18. Satellite image of Northern Territory Australia map. Source www.tomtom.com
19. Garmin BirdsEye Satellite Imagery https://www.ja-gps.com.au/Garmin/birdseye-satellite-imagery/
20. Bureau of Meteorology (2021) 'Climate statistics for Australian locations.' Available at http://www.bom.gov.au/climate/averages/tables/cw_037010.shtml (Accessed 10 July 2021).
21. Slippers, B.; Wingfield, M.J. *Botryosphaeriaceae* as endophytes and latent pathogens of woody plants: Diversity, ecology and impact. *Fungal Biol. Rev.* **2007**, *21*, 90-106. https://doi.org/10.1016/j.fbr.2007.06.002
22. Islam, M.S.; Haque, M.S.; Islam, M.M.; Emdad, E.M.; Halim, A.; Hossen, Q.M.M.; Hossain, M.Z.; Ahmed, B.; Rahim, S.; Rahman, M.S.; Alam, M.M.; Hou, S.; Wan, X.; Saito, J.A.; Alam, M. Tools to kill: Genome of one of the most destructive plant pathogenic fungi *Macrophomina phaseolina*. *BMC Genomics* **2012** 13, 493-508. https://doi.org/10.1186/1471-2164-13-493
23. Burgess, T.I.; Tan, Y.P.; Garnas, J.; Edwards, J.; Scarlett, K.A.; Shuttleworth, L.A.; Daniel, R.; Dann, E.K.; Parkinson, L.E.; Dinh, Q.; Shivas, R.G.; Jami, F. Current status of the Botryosphaeriaceae in Australia. *Australasian Plant Pathol*. **2019**, 48, 35-44. https://doi.org/10.1007/s13313-018-0577-5