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A National Pathway to Market for Dieback-Free Basic Raw Materials: Mitigating the risk of Phytophthora spread from gravel resources using metham sodium

Dieback Working Group

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Company Name: ArborCarbon Pty Ltd

ACN: 145 766 472

ABN: 62 145 766 472

Address: ROTA Trans 1, Murdoch University, Murdoch, WA 6150

Post: PO Box 1065 Willagee Central, WA 6163

Phone: +61 408 907 152

Website: https://arborcarbon.com.au/

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Glossary of Terms

This document uses terminology which may not be consistent with all technical definitions of the term across sectors, particularly regarding assay outcomes. Within this report, the following terms are defined as:

- Sample location: The unit of analysis used in this report. A single Burley cage, containing two (2) pine plugs inoculated with *Phytophthora*, placed at a defined height and lateral distance within a stockpile.
- Baiting: A diagnostic recovery method used to detect viable Phytophthora from a sample by exposing susceptible plant material ("baits") to sample material under conditions that encourage any surviving Phytophthora to infect the bait. In this report, baiting was used as a confirmatory follow-up method for samples that were negative by direct plating. A sample location was classified as negative only when Phytophthora was not recovered by either direct plating or baiting; any recovery by baiting was treated as a positive result.
- Sterilised/Sterilisation: Used in this report only in the assay-defined sense above (negative recovery at the sampling-location level), and only regarding the sterilisation of the *Phytophthora* genus of microorganisms. It is not used to claim absolute sterility of gravel.
- Positive result: *Phytophthora* was recovered from the sampling location by either method (plating or baiting).
- Negative result: No *Phytophthora* was recovered from the sampling location by either method (plating or baiting).
- NA/Missing outcome: No valid outcome was available for a sampling location (e.g., missing/compromised sample). NA outcomes are excluded from percentage calculations and regression models unless stated otherwise.
- Mitigation: refers to the operational reduced risk of the final product post-treatment. Indicates that residual risk can remain even when most sample locations are negative.

Abbreviations

- APVMA – Australian Pesticides and Veterinary Medicines Authority
- BRM – Basic Raw Materials
- C×T – cumulative exposure (concentration × time)
- DBCA – Department of Biodiversity, Conservation and Attractions
- DWG – Dieback Working Group
- GCMS – gas chromatography–mass spectrometry
- ISO/IEC 17025 – General requirements for the competence of testing and calibration laboratories
- LoD – Level of Detection
- MITC – methyl isothiocyanate
- MRWA – Main Roads Western Australia
- NATA – National Association of Testing Authorities (Australia)
- PVC – polyvinyl chloride
- QA/QC – quality assurance / quality control
- TS – test samples (used in the moisture section)
- VHS – Vegetation Health Services (DBCA diagnostic laboratory)

Executive Summary

Phytophthora cinnamomi, along with several other *Phytophthora* species, causes significant dieback and mortality across many Australian natural ecosystems. Roading and infrastructure projects present a major pathway for the unintended spread of these soilborne pathogens, particularly through the movement of basic raw materials (BRM) such as laterite gravel. Developing practical methods to reduce the risk is therefore an important component of broader dieback management strategies in Australia. This report presents the methods, results, and findings from an experimental field trial assessing the efficacy of metham sodium treatment for reducing detectable *Phytophthora* survival within gravel stockpiles. Metham sodium treatment was first demonstrated as effective at the quarry scale in 2019. The present trial builds on earlier laboratory experiments in 2005 and field trials conducted in 2012, 2019, and 2024, incorporating operational refinements developed throughout those earlier studies.

The field trial was conducted between 4 September 2025 and 3 November 2025 at Culford Quarry, North Bannister, Western Australia. Two lateritic gravel stockpiles were constructed with Main Roads Western Australia (MRWA) Specification 501 (Main Roads Western Australia, 2023). A treated stockpile containing 2,117.34 m^3 of gravel received metham sodium during stockpile construction, while a 528.91 m^3 untreated control stockpile was also established. Pine plugs were inoculated with three isolates of *P. cinnamomi* and one isolate of P. multivora, which were distributed throughout both stockpiles across 380 sample locations. The bioassay system provided a deliberately conservative and standardised method for assessing treatment performance under commercial-scale operational conditions.

Gravel moisture content and internal stockpile temperature were monitored throughout the trial to confirm that environmental conditions remained conducive to *Phytophthora* survival and metham sodium activity. Moisture content remained within expected operational ranges throughout the study, while internal stockpile temperatures remained well below reported thermal mortality thresholds for both *P. cinnamomi* and *P. multivora.* These findings support the interpretation that negative recovery outcomes within treated samples were attributable primarily to metham sodium/MITC exposure rather than desiccation or heat-related mortality.

Inoculum plugs were retrieved 59 days after treatment and assessed using direct plating onto *Phytophthora* selective agar, followed by baiting of all negative samples for quality assurance. Within the treated stockpile, 171 of 177 *P. cinnamomi* locations (96.6%) and 56 of 61 *P. multivora* locations (91.8%) yielded no detectable recovery. Adjusted logistic regression confirmed a strong treatment effect, with treated sample locations showing lower rates of positive recovery than untreated controls (OR = 15.89; 95% CI 7.64–36.27; $p < 0.001$).

Intergranular MITC concentrations were mapped throughout the treated stockpile to assess whether local variation in fumigant exposure could explain the small number of positive recoveries. Sensitivity analyses accounting for repeated measurements at individual sampling locations supported the primary statistical findings and indicated that within-location clustering did not materially influence the interpretation of treatment efficacy. The treatment effect remained strong when analyses were refitted using cluster-robust standard errors (OR = 15.89; CR2 95% CI 3.51–71.82; $p = 0.002$). Species identity remained an important predictor, while the lateral distance (horizontal distance from the stockpile edge) effect remained insignificant.

MITC concentrations were vertically stratified within the spray box and spatially variable within the stockpile. While most monitored locations exceeded the proposed exposure threshold, several localised low exposure microzones were identified and were associated with positive *Phytophthora* recoveries, suggesting that variability in MITC exposure may contribute to treatment failure under some operational conditions.

From an operational perspective, the trial demonstrates that metham sodium treatment can reduce the probability of detectable *Phytophthora* survival within gravel under commercial quarry conditions and may be suitable for many operational contexts where some residual risk is acceptable. The residual positive-recovery rate observed in treated material was 3.4% for *P. cinnamomi* (6 of 177 locations positive) and 8.2% for *P. multivora* (5 of 61 locations positive). These findings indicate that the suitability of metham sodium treatment should be considered in relation to management objectives, adjacent environmental values, and acceptable levels of residual risk. In higher-risk ecological or conservation settings, metham sodium treatment should be implemented within a broader, integrated Phytophthora dieback management framework that incorporates hygiene controls, monitoring, and site-specific risk assessment.

1 Introduction

1.1 Background

Phytophthora cinnamomi and *P. multivora* are introduced soil-borne pathogens that kill or weaken many Australian native plants across multiple genera. In Western Australia (WA), it has been demonstrated that disease spread is common where infected gravel or soil is moved for road construction (Hardy et al. 2001). This has created a high demand for uninfested ("dieback-free") gravel that is difficult to guarantee without a reliable treatment and verification process (Davison et al. 2019). The Phytophthora Dieback Management Manual (2020) (Department of Biodiversity, Conservation and Attractions 2020) sets out operational requirements for managing dieback risk during disturbance activities, including guidance on when uninfested basic raw material (BRM), such as gravel, must be used based on the dieback status of roads and adjacent areas. Key requirements include:

- Classification of areas and road surfaces according to *P. cinnamomi* infestation status
- Restriction of gravel movement from infested to uninfested areas
- Use of uninfested BRM in dieback-free areas and in high-value conservation areas
- Implementation of hygiene and verification procedures to prevent pathogen spread.

Phytophthora cinnamomi is listed as a Key Threatening Process under the *Commonwealth Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) due to its significant impacts on Australia's biodiversity. It is also managed within state biodiversity conservation legislation and is a primary focus of state-based dieback management policies and operational hygiene frameworks in several jurisdictions (often centred on *P. cinnamomi*, while recognising risks from other *Phytophthora* species).

Phytophthora multivora is also widespread, has a broad host range, and is associated with substantial ecological impacts. *Phytophthora multivora* was included in this work alongside *P. cinnamomi* because it differs biologically (being homothallic) and produces long-term survival spores (oospores), which may persist longer than asexual survival structures under some adverse conditions.

Metham sodium is a common biocide used within the horticulture industry, which, when mixed with water, decomposes into a gas called methyl isothiocyanate (MITC). MITC is the component that kills, and it has been demonstrated to effectively reduce the risk of *P. cinnamomi* spread in treated BRM when applied properly.

This project tests whether metham sodium treatment can reliably reduce the risk of spread of *Phytophthora* in road-building gravel at a commercial scale, with the end goal of validating the use of metham sodium to produce a cost-effective BRM source with a significantly reduced likelihood of spreading *Phytophthora* into new areas.

1.1.1 Overview of previous studies

Research into metham sodium treatment of gravel has progressed through Curtin University and Main Roads Western Australia (MRWA) investigations conducted between 2005 and 2012 (Davison et al. 2019), followed by an industrial-scale trial in 2019 (Williams and Eslick 2019). A further large-scale trial in 2024 was deemed inconclusive due to low survival of *Phytophthora* in the untreated control stockpile (unpublished; data not provided), limiting the interpretability of treatment performance. These previous trials established the technical and regulatory foundation for gravel fumigation using metham sodium, and informed progressive refinements which were operationalised by MRWA, but remained untested. The present (2025) trial was

commissioned to strengthen statistical confidence in treatment efficacy under commercial conditions while incorporating lessons learned from previous trials. Key methodological differences between the 2019, 2024 and 2025 trials are summarised in Table 1.

Key points from previous work:

- Research by Curtin University and MRWA demonstrated that metham sodium can significantly reduce *P. cinnamomi* and *P. multivora* survival in a laboratory and small-scale field trial setting (Davison et al. 2019).
- These results supported Nufarm Australia's successful APVMA label amendment to include metham sodium treatment of gravel for *Phytophthora* species, including an approved application rate (80 mL/m³), buffer requirements, and a quarantined withholding period post-treatment (APVMA Approval No. 34049/108668).
- The 2019 trial at Culford Quarry resulted in the sterilisation of 94.2% of samples. These findings enabled MRWA's move toward commercial use of metham sodium-treated gravel from 2020, including development of SOPs incorporating process improvements identified during the 2019 trial operation and presumed to further reduce the risk of *Phytophthora* survival.
- Western Australian Department of Biodiversity, Conservation and Attractions (DBCA) requested further evidence on the efficacy of these improvements prior to approving the use of metham sodium-treated gravel on the conservation estate.
- The 2024 trial aimed to address this knowledge gap by repeating the 2019 experiment at a larger scale and using methods and improvements stipulated in the MRWA SOPs. This trial was unfortunately compromised by low *P. cinnamomi* and *P. multivora* survival in the control pile (35 positive out of 140 = 25% survival), limiting the interpretability of the trial results.
- Intergranular monitoring during the 2024 trial recorded lower-than-expected in-pile MITC concentrations (ppm), prompting an investigation into potential loss pathways during transfer and application. Subsequent testing demonstrated that metham sodium begins converting to MITC during dilution and transfer through the 121 m delivery hose, with measurable losses occurring at the spray box/nozzles prior to contact with gravel. Based on these findings, the target application rate was increased (from 80 mL/m³ to 160 mL/m³) to compensate for losses and maintain the intended in-pile dose.
- The 2025 trial presented in this report repeats the 2024 trial stockpile construction and monitoring methodology, while incorporating learnings from the 2024 operation.

Table 1. Summary of key methodological differences between metham sodium gravel trials in 2019, 2024, and 2025.

Aspect	2019	2024	2025	Reason for change
Weather conditions	Treatment period, 16–23 September 2019: maximum temperature 14.3–23.6 °C; minimum temperature −0.8–8.2 °C; rainfall 10.8 mm on 19 September.	Not reported in the 2024 results analysis: ➢ September 2024 (Wandering, 010917 – Bureau of Meteorology) – Rainfall: 24.6 mm total; Temperature: min −0.6 °C, max 27.2 °C. ➢ October 2024 (Wandering, 010917 – Bureau of Meteorology) – Rainfall: 26.8 mm total; Temperature: min 1.0 °C, max 34.5 °C. ➢ November 2024 (Wandering, 010917 – Bureau of Meteorology) – Rainfall: 10.6 mm total; Temperature: min 0.9 °C, max 35.5 °C.	Withholding period, September–October 2025: September rainfall 83.6 mm across 17 rain days, with maximum daily rainfall of 23.8 mm on 6 September; October rainfall 32.2 mm, with maximum daily rainfall of 10.4 mm on 21 October.	Observed conditions
Volume of gravel treated	1,250 m^3	1,900–2,400 m^3	2,117.34 m^3	Increased scale to imitate commercial treatment protocols, namely the use of a bund to limit gravel separation during stockpile construction, which may limit MITC exposure.
Water seal application	Not applied	Applied	Not applied	Not required in 2025 due to already high gravel moisture and high rainfall during the withholding period.
Bunds around stockpile edge	No	Yes	Yes	Adopted within MRWA stockpile construction SOPs to eliminate the issue of segregation of coarse gravel at the toe of the stockpile observed in 2019, which resulted in lower MITC retention, and a higher risk of *Phytophthora* survival.
150 mm gravel "floor"	No	Yes	Yes	Adopted within MRWA stockpile construction SOPs, allowing a sacrificial layer of gravel to remain post-treatment to further mitigate *Phytophthora* risk.
Stockpile configuration	Straight piggyback stockpiles	Three overlapping crescents	Three overlapping crescents	Adopted within MRWA stockpile construction SOPs, to reduce segregation of material observed in 2019, and align with modern gravel processing methods and machinery.
Inoculum incubation	12 weeks	~6 weeks	146 days ~19.5 weeks	Extended in 2025 after low inoculum survival in 2024, and to

Aspect	2019	2024	2025	Reason for change
time within flasks prior to deployment				better match the incubation period of 8–10 months of the Davison *et al* (2019) study.
Isolate selection	Mixed isolates (*P. cinnamomi*, *P. multivora*) see Williams and Eslick (2019)	Same as 2019	Updated isolates (Table 2)	Isolates selected for viability as demonstrated below. *Phytophthora cinnamomi* was included because it is the primary species targeted by Phytophthora dieback management frameworks in Western Australia and is listed as a Key Threatening Process under the EPBC Act. *Phytophthora multivora* was included because it is widespread, has recognised ecological impacts, and differs biologically from *P. cinnamomi*, including its homothallic reproductive strategy and capacity to produce oospores.
Passage of *Phytophthora* isolates through Granny Smith Apples	No	No	Yes	To increase pathogenicity and fitness of the *Phytophthora* isolates and to make them physiologically similar when removed from storage.
Pre-trial inoculum validation	Growth on a *Phytophthora* selective medium	Growth on selective medium	Selective & non-selective media plus confirmation of the formation of resting structures.	Additional Quality Assurance, introduced after 2024 control failures.
Examination of colonised plugs for the presence of mycelia and survival structures	No	No	Yes	Plugs examined microscopically after clearing and staining thin sections of plugs from each isolate for evidence of mycelia and survival structures.
Sample tube insertion	Manual placement during pile build	Inserted into pre-formed holes	Inserted into pre-formed holes	Improved safety and precision necessary when working at larger scales.
Sample tube slotting	40 × 2 mm slots near surface	2 mm slots on both sides of the insertion tubes	Same as 2024	Improved MITC gas transfer while maintaining pipe strength.
Sample pipe configuration	Individual pipes with balloon plugs	Connected modular 1 m sections, with blockers between 1 m sections	Same as 2024	Reduced dilution of MITC along pipe length.
Burley cages	Powder-coated metal	Mixed metal and plastic	Single plastic mesh type	Plastic cages chosen due to concern that leachates from the metal cages

Aspect	2019	2024	2025	Reason for change
				might be toxic or inhibitory to *Phytophthora.*
Intergranular MITC testing	No	Yes	Yes	Added to assess MITC permeation patterns and trends throughout the stockpile.
MITC testing near shroud	No	No	Yes	Added to assess MITC loss at the point of application.
Metham sodium application rate	Rates ranged from 52–120 mL/m³	Dose to gravel (label rate): 80 mL metham sodium per m³. Spray-box set rate: 143.6 mL/m³ to compensate for measured losses prior to gravel contact (shroud/nozzle volatilisation)	Dose to gravel (label rate): 80 mL metham sodium per m³. Spray-box set rate: 173.1 mL/m³ to compensate for measured losses prior to gravel contact (shroud/nozzle volatilisation)	Adjusted to account for MITC loss at the point of delivery in the shroud. Therefore, in 2025, the rate was increased to ensure an accurate delivery rate into the gravel of 80 mL/m³.

2 Aim

This project aims to provide a scientifically robust dataset that supports land managers and policy makers to consider the adoption of a novel, cost-effective, and sustainable method of obtaining low risk, BRM for civil construction. This trial forms part of the Dieback Working Group (DWG) led "National Pathway to Market for Dieback-Free BRM" Commonwealth-funded project, which aims to support wider market availability of treated materials at a national scale.

Specifically, the project quantifies the efficacy of metham sodium treatment in mitigating *P. cinnamomi* and *P. multivora* in gravel relative to an untreated control stockpile, while building on methodological learnings from previous trials conducted in 2019 and 2024.

The present trial aims to address the following research questions:

1. To what extent does metham sodium treatment reduce the probability of detecting *P. cinnamomi* and *P. multivora* in gravel?
2. When MRWA operational refinements from the 2019 field trial are implemented while otherwise using the same core experimental design, does the efficacy of treatment improve from the 94.2% sterilisation rate observed in 2019?

3 Methods

3.1 Experimental design overview

This trial compared an experimental, metham sodium-treated gravel stockpile to an untreated control stockpile (Figure 1). The treated stockpile was built using 2,117.34 m^3 of gravel under commercial operational conditions. Stockpiles were constructed using a conveyor stacker with metham sodium applicator located at the top of the stacker conveyor (Figure 1). This construction approach reflects standard quarry stockpiling practice and provides a consistent operational basis for comparing the treated and untreated control stockpiles. A smaller untreated control stockpile was constructed using 528.91 m^3 of gravel from the same source, without fumigant application. To assess treatment performance, *Phytophthora*-inoculated pine plug samples were installed in both stockpiles. These samples were housed within slotted PVC sampling tubes and positioned within the stockpiles at predefined depths and lateral distances.

Sample locations were arranged throughout the experimental and control stockpiles to assess potential spatial variation in *P. cinnamomi* and *P. multivora* mitigation throughout the stockpile profile. Detailed schematics of stockpile geometry, sampling layouts, and modular pipe design for both the treated and control stockpiles are provided in Figure 7 and Figure 8. The overall methodology is summarised below (corresponding section numbers in brackets):

- Experimental design and layout: treated vs untreated control stockpiles; sampling grid by depth and lateral distance (3.1).
- Inoculum development and quality assurance: isolate selection, pine plug production, incubation, and validation prior to field deployment (3.2).
- Gravel preparation and stockpile construction: gravel handling, water-source screening, moisture management, and stockpile geometry (3.3).
- Monitoring during fumigation: internal temperature monitoring, ambient meteorology, environmental/perimeter MITC monitoring, and intergranular MITC monitoring (3.4).
- Metham sodium application: application system, dose, calibration, and operational controls during treatment (3.5).
- Installation of inoculum and sampling infrastructure: placement of inoculated plugs in Burley cages within modular PVC sampling tubes, including installation method and traceability (3.6).
- Post-treatment recovery and viability testing: plug retrieval, handling and transport, plating and baiting workflows, and outcome definitions (3.7).
- Data handling and statistical analysis: treatment efficacy models, adjusted spatial and biological covariate models, and sensitivity analyses (4).

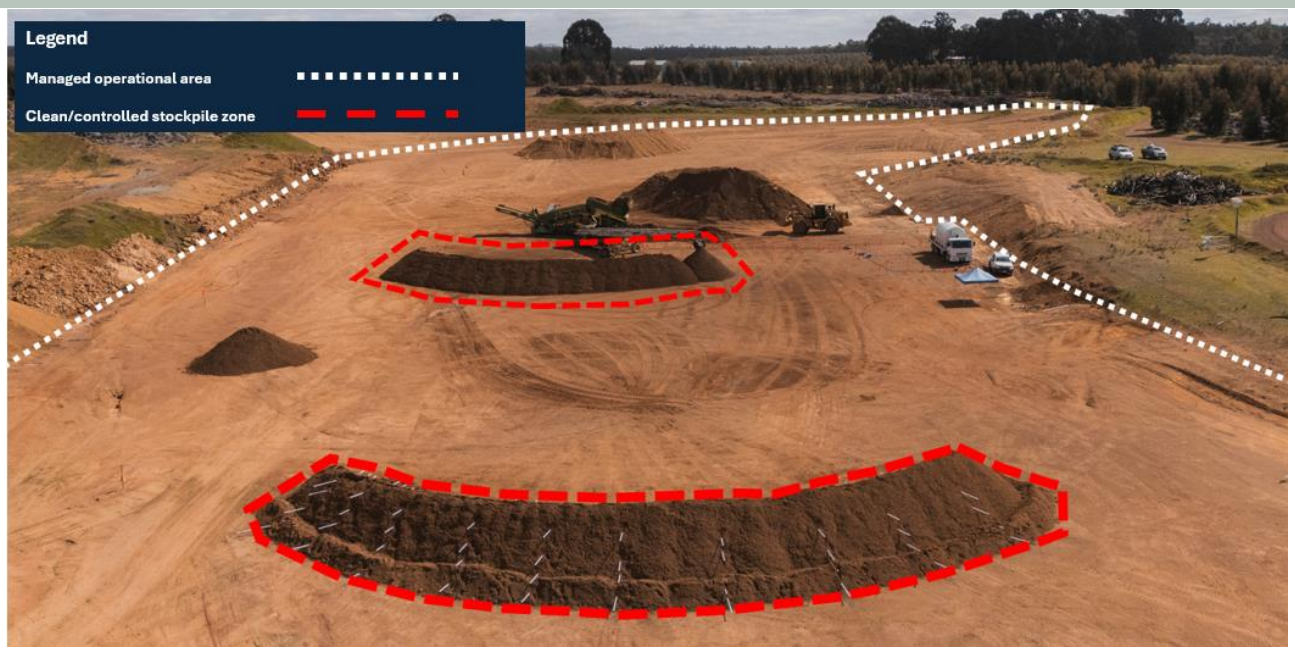


Figure 1. Drone image of the treatment site at the Culford Agri Industry Precinct, North Bannister, Western Australia. The white dotted boundary indicates the managed operational zone used to control site access and movement. The red dashed boundaries indicate the clean construction zones for the experimental stockpiles, which were managed under uninfested pit protocols. The control stockpile (under construction) is shown in the foreground, and the treated stockpile is shown in the background.

This study follows a nested sampling design, centred around using pine plugs inoculated with *Phytophthora* and distributing them throughout the stockpile in a three-dimensional grid distribution. Sample distribution was planned to allow an assessment of fumigant performance via the plug bioassay throughout the stockpile profile. To measure horizontal variation in treatment efficacy, pipes were spaced around the footprints of the stockpiles. To measure any vertical variation in efficacy of treatment, each group (1–20) contained PVC tubes installed parallel to the ground at four different heights (A, B, C, and D). This sampling design resulted in a total of 240 sample locations in the treated stockpile, equating to 480 inoculated plugs (two plugs per location).

This sampling design ensures that:

- Plugs are protected during handling and stockpile compression.
- Plugs can be reliably placed at defined heights and lateral distances via insertion of the sampling infrastructure.
- MITC diffusion to the inoculum location is possible via the slotted design of the sample tubes.
- Collection of intergranular MITC data and intra-stockpile temperature data is made possible, through inclusion of nylon sampling lines and HOBO data loggers to the sampling infrastructure.
- Independence between samples within the same tube is maintained through airtight separators between modules.
- Plugs can be safely and reliably retrieved at trial completion.

Four isolates of *Phytophthora* were used to inoculate the plugs (Table 2); 3 different isolates of *P. cinnamomi* and 1 isolate of *P. multivora.* To achieve this, plugs were installed using a modular system of slotted 40 mm CL18 PVC pipe lengths (modified from Davison, 2019) of 1 m per module and housed within these modules in 82 mm long by 32 mm wide Burley cages, colour coded by isolate. Slots cut into PVC pipes were 10–12 mm long × ~2 mm wide, arranged in two opposing rows (180° offset) around the pipe circumference to

balance gas exchange and structural integrity under stockpile compression (Figure 2 a.). Each Burley cage (Figure 2 b.) housed two inoculated pine plugs (Figure 2 c.) of the same isolates and was installed within the PVC tubes. To ensure the Burley cages remained in position within the tubes, a screw was drilled through the PVC pipes on either side of the cages.

Table 2. Phytophthora *isolates, species, mating types, and origins used as inoculum, and the colours used to designate each isolate on the Burley cages and bags.*

Isolate number	Species	Mating Type	Origin (Location, host, isolation date)	Burley cage colour
37439	*P. cinnamomi*	A1	Cape Riche, *Banksia cuneata*, 1/2/2018	Pink
48828	*P. cinnamomi*	A2	Wilga block, *Leucopogon capitellatus*, 3/2/2025	Yellow
9701	*P. cinnamomi*	A2	O'Neill Block, *Banksia grandis*, 4/9/2001	Blue
37571	*P. multivora*	Homothallic	Cooljarloo, *Banksia* species, 26/2/2018	Green

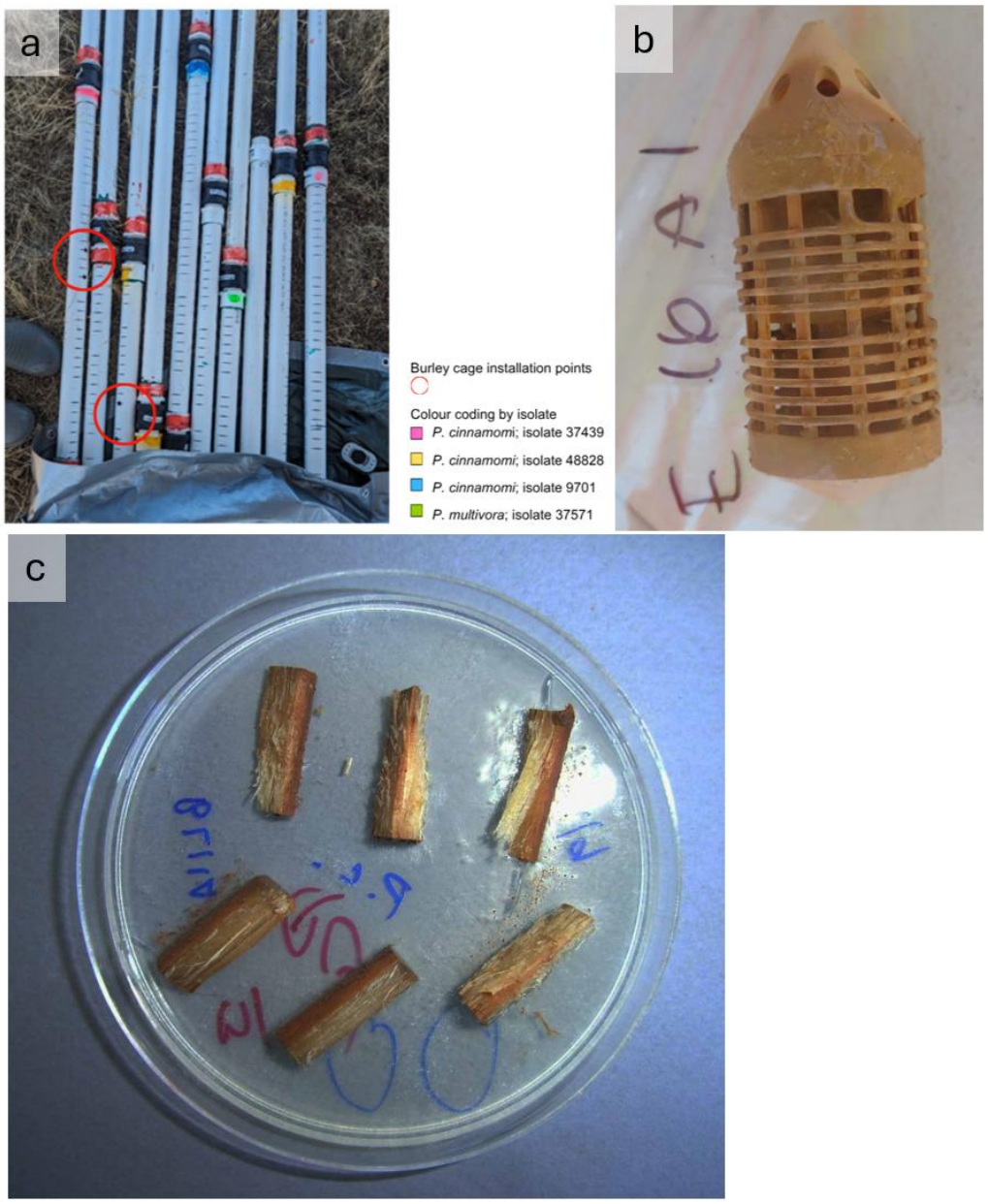


Figure 2. (a.) Slotted PVC sampling tubes, colour coded by isolate, red circles indicate where screws to hold Burley cages in place are visible. (b.) Labelled perforated Burley bait cage used to contain two Phytophthora *colonised inoculum plugs. Each cage was uniquely labelled and installed within a modular PVC sampling tube at predefined depths and locations within the gravel stockpile. The perforated design allowed exposure to intergranular moisture while preventing direct contact with surrounding gravel. (c.) Pine plugs split into sections for confirmation of Phytophthora colonisation prior to placement into labelled perforated Burley bait cages for installation within the PVC sampling tubes.*

Treated stockpile sample distribution

The footprint of the treated stockpile was approximately 60 m in length by 20 m in width, with a total stockpile volume of 2,117.34 m^3.

To measure any vertical variation in efficacy of treatment, tubes containing a total of 240 sample locations were installed in 20 replicate groups of PVC pipes, installed parallel to the ground at four heights (A=0.5 m,

B=1.0 m, C = 1.75m, D=2.5m). Due to the geometry of the stockpiles, pipes at each height were comprised of a different number of modules (and therefore differed in length and number of sample locations).

In the treated stockpile, each group (1-20) contained 12 sample locations total across levels A to D (Figure 7):

- A: comprised of 5 modules, inserted at 0.5 m above ground level, containing 4 sample locations per pipe.
- B: comprised of 4 m modules, inserted at 1.0 m above ground level, containing 3 sample locations per pipe.
- C: comprised of 5 modules, inserted at 1.75 m above ground level, containing 4 sample locations per pipe. Includes a location aligned with the inter-peak gully (Figure 7).
- D: comprised of 2 modules, inserted at 2.5 m above ground level, containing 1 sample location per pipe.

Control stockpile sample distribution

In the 528.91 m³ control stockpile, sampling infrastructure was also installed as 20 replicated groups distributed around the stockpile footprint of approximately 60 m in length by 8 m in width (Figure 8). A total of 140 sample locations were installed in total.

In the control stockpile, each group (1-20) contained 7 sample locations total across levels A to D (Figure 8):

- A: comprised of 3 modules, inserted at 0.5 m above ground level, containing 2 sample locations per pipe.
- B: comprised of 3 modules, inserted at 1.0 m above ground level, containing 2 sample locations per pipe.
- C: comprised of 3 modules, inserted at 1.75 m above ground level, containing 2 sample locations per pipe.
- D: comprised of 2 modules, inserted at 2.5 m above ground level, containing 1 sample location per pipe.

3.2 Development and validation of the inoculum

3.2.1 Vegetation Health Service Laboratory

Pine plugs were inoculated with *Phytophthora* isolates and quality-checked by the Vegetation Health Services (VHS) laboratory, a dedicated *Phytophthora* diagnostic laboratory operated by the DBCA, based at the main DBCA office in Kensington (Perth, WA). VHS also completed the final re-isolation and verification steps for the 2019 trial following the methods described by Williams and Eslick (2019).

Sample handling and tracking followed standard laboratory procedures. Quality control and release of inoculum for field deployment were overseen by the laboratory manager, including verification of inoculum viability (via re-isolation on appropriate media) and checks for contamination prior to installation.

3.2.2 Species and isolates selection

Phytophthora isolates were sourced from the VHS culture collection. Four *Phytophthora* isolates were used as inoculum in this trial: *Phytophthora cinnamomi* (isolates 37439, 48828, 9701) and *P. multivora* (isolate 37571) (Table 2). Where possible, isolates were selected to maintain continuity with previous metham sodium gravel trials (Williams and Eslick 2019). Isolate identification was validated through morphological assessment at the (VHS) and confirmed by molecular sequence-based identification at the Centre for Biosecurity and One Health, at the Harry Butler Institute, Murdoch University.

Isolate viability checks

Prior to field deployment, isolates were reinvigorated by inoculating Granny Smith apples (*Malus domestica*), allowing lesions to develop and then reisolating using established *Phytophthora* culturing approaches (e.g., apple-based pathogenicity/colonisation checks and standard re-isolation methods), supporting inoculum viability and integrity (Popp et al. 2019). Inoculation of all isolates into the apples at the same time helped ensure that they were in a similar physiological state after being stored *in vitro* for differing periods of time (Tooley 1988).

The *P. cinnamomi* isolates DP51 and 37571 used for the 2019 and 2024 trials failed to colonise the apples and were replaced with viable isolates from the same mating type and geographic area for the 2025 trial.

3.2.3 Preparation of inoculated pine plugs

Pine plug inoculum was produced using the approach described by Davison et al. (2019) with the four *Phytophthora* isolates (Table 3). Debarked *Pinus radiata* plug material was sourced from the Nairn Pine Plantation, adjacent to the Southwestern Highway, south of Quinninup (UTM Zone 50: E 430700, N 6186250; map-derived). Stems were cut using sterilised long-handled secateurs and either processed into plugs on site under shade beneath mature pine trees or transported as 2–3 m stems to a Manjimup workshop (~30 min). Once processed, harvested stems/plugs were transported to VHS in insulated boxes. Debarked wood plugs were rinsed in tap water, soaked for 24 hours in de-ionised water in 1–2 L flasks, then sterilised by autoclaving at 121 °C for 30 min in flasks containing a shallow sterile water layer. Following autoclave sterilisation, flasks were drained of all residual water prior to inoculation.

Pine plugs were inoculated with actively growing *Phytophthora* cultures on pea and/or cornmeal agar (CMA), poured at ~20 mL per 90 mm Petri plate (as described in Davison et al. 2019). Each flask containing pine plugs was inoculated with a fully colonised plate of a 7-day-old culture for both pea and CMA agar. The colonised agar was cut into approximately 1 cm² cubes, which were added to the flasks. Each flask was gently agitated to evenly distribute the agar sections throughout the pine plugs. Flasks were stoppered with breathable cotton-wool bungs wrapped in Miracloth (Merck Millipore) to form an easily removable porous seal, and the bung/neck assembly was covered with aluminium foil to reduce the risk of contamination during sterilisation and handling (Versalovic 2011). No additional moisture was added during incubation, consistent with methods used in previous gravel fumigation trials (Davison et al. 2019).

Following inoculation, pine plugs were incubated under temperature-controlled laboratory conditions to promote uniform colonisation prior to field deployment. Plugs were incubated from 17 April 2025 to 31 August 2025 (~19.5 weeks). The flasks were gently shaken again on 28 April 2025 and on 13 May 2025, to ensure even mycelial colonisation throughout the plugs in each flask. Where fungal or bacterial contamination growth occurred during inoculum production, all plugs within the affected flasks were discarded and not used for field deployment. From the time of transport to the Culford gravel pit on the 31 August 2025, until insertion into the stockpiles, the plugs were stored at room temperature in a sterilised insulated box. The number of inoculated plugs produced per isolate and the total number of plugs produced are shown in Table 3.

Table 3. Number of inoculated pine plugs produced for field deployment for different Phytophthora *isolates and mating types.*

***Phytophthora* species**	**Isolate Number**	**Mating types**	**Number of baits produced by the lab**
Phytophthora cinnamomi	37439	A1	990
Phytophthora cinnamomi	48828	A2	1010
Phytophthora cinnamomi	9701	A2	1000
Phytophthora multivora	37571	Homothallic	1000
Total pine plugs			4330

3.2.4 Sample viability and screening for contaminants

Due to the high proportions of failed samples in the 2024 trial, an additional set of strict laboratory quality assurance checks and hygiene protocols was implemented for this project. The DWG has prepared method statements to facilitate the replication of this experiment in future research projects (email: hello@dwg.org.au).

Quality assurance and hygiene management

To ensure the maturity of the inoculum through the presence of resting structures including chlamydospores, oospores, stromata (dense aggregations of hyphae and hyphal swellings) were formed in the inoculum plugs, monitoring by microscopic assessment (Olympus BH2 compound microscope at 400 and 100x magnification) was performed from July to August 2025. Thin sections were prepared using a razor blade from three plugs per isolate (n=3), stained and cleared with lactoglycerol and aniline blue to visualise *Phytophthora* mycelium and resting structures. Chlamydospores and hyphae were successfully observed for all three *P. cinnamomi* isolates, and oospores and hyphae in *P. multivora.*

Viability and cleanliness were confirmed at multiple time points during incubation, and immediately prior to field deployment. On 26 May 2025, four plugs were sub-sampled from each of the four isolates (16 plugs total) and plated for viability assessment. No growth was observed from the selected flask of Isolate 37439, consistent with the absence of visible mycelial growth; all other isolates showed strong growth. On 21 July 2025, four plugs were sub-sampled from Isolates 48828, 9701 and 37571 (12 plugs total), with all yielding *Phytophthora* growth. On 4–5 August 2025, four plugs were taken from a different flask of Isolate 37439 and confirmed viable (4 plugs total). Additional confirmatory plating of two plugs per isolate (8 plugs total) was undertaken in early August and demonstrated active growth from all isolates. Immediately prior to field deployment (31 August 2025), four plugs were randomly selected from the prepared batch and confirmed viable. In each assessment, plugs were surface-sterilised (70% ethanol for 30 seconds, rinsed twice in sterile water), split longitudinally, cut into 2–3 cm sections, and plated onto *Phytophthora*-selective agar (NARPH). Plates were monitored for up to 14 days for *Phytophthora* growth.

Field deployment conditions

For field deployment (31 August 2025), the inoculum plugs were transported to Culford Quarry using plastic bags in insulated boxes. The Burley cages were colour coded with spray paint to designate the different isolates (Table 2) and sterilised with 70% ethanol prior to two plugs of a single isolate being inserted per Burley cage. For deployment and analysis, samples were grouped by stockpile, species, and isolate.

3.3 Site management

3.3.1 Clean on Entry Zones and on-site *Phytophthora* hygiene

Culford Quarry is a secure gated property, with no unauthorised access allowed, which may compromise hygiene management. To adhere to operational hygiene conditions outlined by the DBCA 'Phytophthora Dieback Management Manual' (2020). The project was conducted using a strict "Arrive Clean, Leave Clean" protocol for all approved access at the quarry site. All vehicles, machinery, and footwear were required to be free of all soil and plant material before entering the site, with manual brushing or high-pressure water used at designated clean on entry inspection points (Figure 3). Activities were scheduled preferentially during dry soil conditions to minimise the risk of pathogen introduction via vehicles and equipment. Clean down

was performed on hand tools and footwear using a 70% methylated spirits solution, ensuring all surfaces were thoroughly treated before moving between management zones.

Figure 3. Drone image depicting site layout at the Culford Agri Industry Precinct, North Bannister, WA. The dotted white line delineates the operational zone, within which all access was strictly managed for dieback hygiene. Within the operational boundary, the control stockpile (i), treated stockpile (ii), source material (iii), and chemical treatment truck (iv) can be observed. The treated and control stockpile construction zones are demarcated by a red dashed line and were managed in accordance with uninfested pit management protocols (DBCA, 2020).

3.3.2 Water source pathogen screening

Fresh water used for gravel moisture conditioning and metham sodium mixing was sourced from a sealed, lined dam constructed on site (Figure 4). The dam water was screened for water-borne *Phytophthora* in 2024 using *Quercus suber* (oak) and *Hedera helix* (ivy) baits (Burgess et al. 2021), deployed from 17 October 2024 to 24 October 2024 (Figure 4). The samples were submitted to DBCA VHS on retrieval (VHS sample IDs 48167–48170). Results were reported on 22 November 2024. *Phytophthora thermophila* was detected in three of four bait samples. One sample was reported as negative for all *Phytophthora* species

Phytophthora thermophila (Clade 6 species) is often found in inundated soils or riparian zones in Western Australia. It is self-sterile and survives through the production of sporangia, chlamydospores and zoospore cysts. Although, common in water, it has been associated with scattered mortality in several native plant species but frequently co-occurs with other more pathogenic *Phytophthora* species (Belhaj et al. 2018). Noting that pathogenicity studies are limited, this species is considered an opportunistic or minor pathogen and is not managed under any government Act. As such, the absence of *P. cinnamomi*, *P. multivora* or any other terrestrial *Phytophthora* species met the criteria for use of this water source under the experimental conditions without UV or other treatment.

Figure 4. Water storage dam used for gravel moisture conditioning and metham sodium mixing. The sealed dam was screened for water-borne Phytophthora *in October 2024 prior to use in the trial. The arrow denotes a bait float, attached to a length of fishing line, used to capture potential* Phytophthora *species within the dam. This screening was not repeated in 2025.*

3.3.3 Moisture management

Metham sodium catalyses into the gaseous MITC when mixed with water, and the speed of this conversion is impacted by the relative amount of water to metham sodium dose (dilution rate). As an additional quality assurance measure stemming from the unsuccessful 2024 field trial, gravel moisture was measured both pre-construction (28 August 2025; ASM: MC25-02241), and during stockpile dismantling/plug retrieval (3 November 2025; Control: ASM:MC25-02849; Treated: ASM:MC25-02850). Samples were collected and analysed by a NATA-accredited laboratory (in accordance with WA110.1).

Gravel moisture prior to stockpiling ranged from 7.5–8.3% (n=4, mean =8.0%). During dismantling, gravel moisture content ranged from 8.6–9.7% in the control stockpile (n=12, mean =9.2%) and 8.1–10.8% in the experimental stockpile (n=12, mean =9.6%). Moisture content summary statistics by sampling stage and stockpile are presented in Table 4. Additional results are provided in Appendix A: Gravel moisture assessment, and the result certificates provided in Appendix B: Gravel moisture data sheets.

Table 4. Gravel moisture summary statistics by sampling stage and stockpile. Values summarise laboratory-determined moisture content (%) for each stage/stockpile.

Sampling stage / stockpile	Range (%)	Mean moisture (%)	n
Baseline (source gravel pre-construction)	7.5–8.3	8.0	4
Control stockpile (dismantling/plug retrieval)	8.6–9.7	9.2	12
Treated stockpile (dismantling/plug retrieval)	8.1–10.8	9.6	12

3.3.4 Gravel screening and handling

Gravel was supplied and handled by Culford Quarry (Culford Agri Industries), located at 6364 Albany Highway, North Bannister, Western Australia, with all processing and stockpile construction undertaken on site. The source material was lateritic pedocrete gravel, commonly used for road construction. Gravel was homogenised and screened to Specification 501 standards (Main Roads Western Australia 2023). The overburden was removed prior to stockpiling to meet MRWA 501 Specification, and to allow for accurate calculations of metham sodium dosage by volume. Construction of the untreated control stockpile was completed on 1 September 2025, and construction of the metham sodium-treated stockpile was completed between 2 and 4 September 2025. Material was transferred to the construction area using a Telestack conveyor stacker. Gravel feed to the screen and stacker was regulated via the screen hopper and loader cycles to maintain a constant volume of material during stacking (Figure 5).

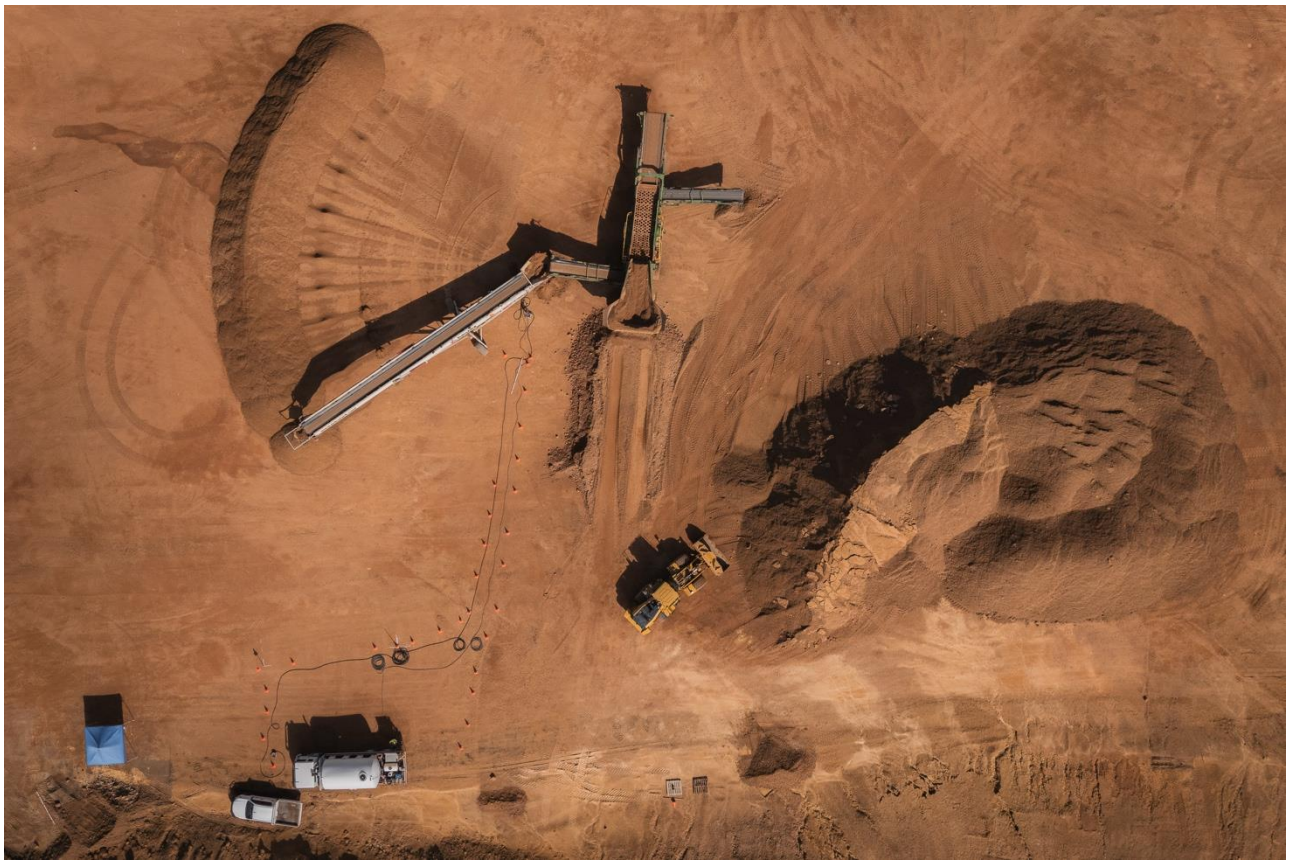


Figure 5. Aerial view of gravel processing and stockpile construction at Culford Quarry, North Bannister, Western Australia, captured by drone on 2 September 2025. The Telestack conveyor stacker and partially constructed stockpile are shown in the upper left of the image, with the metham sodium dosage truck and chemical delivery lines visible in the lower left. Unscreened gravel to be loaded into the hopper and treated is visible centre right.

3.4 Monitoring during fumigation

Monitoring was undertaken to confirm that fumigation conditions were suitable for *Phytophthora* control and to support interpretation of the bioassay results. Monitoring included: (i) measurement of temperature and weather conditions during application and the withholding period, (ii) independent monitoring of MITC in air at the point of application and in the surrounding environment, and (iii) monitoring of MITC within the stockpile gravel (intergranular gas) to confirm fumigant penetration (Du et al. 2026). Bioassay recovery testing and post-treatment viability assessment are described separately in section 3.7.

3.4.1 Temperature monitoring

Meteorological conditions (ambient)

Meteorological conditions were recorded as part of the independent MITC monitoring program to support the interpretation of MITC generation, dispersion, and off-site movement during the application and monitoring period (Du et al. 2026). Recorded variables included ambient temperature and wind conditions, which were used to contextualise spray-box, in-stockpile, and environmental MITC measurements. Complete meteorological datasets and interpretation are reported in the accompanying MITC field monitoring report (Du et al. 2026). Ambient meteorological conditions were monitored during the metham sodium withholding period to support the interpretation of MITC generation, dispersion, and dissipation, using a site digital weather station (Nylex, Australia) and rainfall records from the Bureau of Meteorology Wandering station (BoM station 010917; WMO 95640), the nearest relevant BoM station to the Culford site. September 2025 was comparatively wet, with 83.6 mm recorded across 17 rain days; the largest daily rainfall event (23.8 mm) occurred on 6 September, coinciding with the start of the withholding period. In contrast, October 2025 was drier, with 32.2 mm of rainfall concentrated into a few events (a maximum of 10.4 mm on 21 October).

Stockpile internal temperature probes

Internal temperature range monitoring within the stockpiles was undertaken using HOBO MX2201 data loggers (Onset Computer Corporation) installed in the PVC pipes at predefined locations within the treated and untreated control stockpiles during the application and withholding period (Treated n = 6, Control n = 12). Given the low survival of control samples in the 2024 trial, it was considered essential to verify that internal stockpile temperatures remained within a range consistent with *Phytophthora* survival and growth (i.e., well below sustained high-temperature conditions known to inactivate *Phytophthora* propagules). A total of 6 data loggers were deployed in the treated stockpile (1C3, 5C1, 7B3, 9B1, 17B2, 18A3) and 12 loggers in the control stockpile (1A1, 1D1, 3B1, 6A1, 8C2, 9C2, 10B2, 11D1, 14C2, 15C1, 17C2, 18C1).

Loggers recorded temperature at 5-minute intervals with timestamps in AWST from 5 September 2025 to 3 November 2025, with slight variation in probe start times due to sequential configuration and deployment (treated loggers commenced on 2 September 2025, with two probes commencing on 4 September 2025). Logger data was downloaded at the end of the monitoring period and exported as Excel workbooks. A total of 16,992 observations per data logger were collected for analysis. Time series were screened for completeness and obvious artefacts (e.g., flat lines, dropouts, or implausible spikes). No interpolation was applied, and missing values were retained as missing. For each probe location, the following descriptive statistics were calculated over the analysis window: start time, end time, number of observations (n), mean temperature (°C), standard deviation (SD), minimum (°C), and maximum (°C).

3.4.2 Environmental MITC monitoring

Environmental air monitoring was undertaken to measure MITC concentrations around the stockpile during and after application and to assess potential off-site movement, under site conditions (Du et al. 2026). Monitoring was conducted in four directions (North, West, South and East) at set distances of 1, 5, 10, 50, 100, 150 and 200 m from the point of application. Air samples were collected as grab samples into 1 L Tedlar bags and analysed by Murdoch University's post-harvest biosecurity and food safety laboratory under ISO/IEC 17025-aligned procedures (Du et al. 2026). Results are reported in the accompanying MITC field monitoring report (Du et al. 2026) and are used in this report as supporting evidence for fumigant behaviour during the trial. The analytical limit of detection (LoD) for MITC was 0.02 ppm; results reported below the LoD were treated as non-detections.

3.4.3 Intergranular MITC monitoring

Intergranular MITC monitoring was undertaken to confirm fumigant penetration within the gravel matrix and to support the interpretation of the *Phytophthora* bioassay outcomes (Du et al. 2026). Monitoring locations were designed to align as closely as practicable with the bioassay sampling framework, with the nylon lines used for intergranular sampling attached to the outsides of the PVC sample pipes (Figure 6). MITC monitoring locations were randomly assigned throughout the stockpile (Du et al. 2026), and are depicted spatially against the sample results in Appendix C (Figure 10). A total of 36 probes were installed in the treated pile, 12 probes at height A (0.5 m), 9 probes at height B (1 m), 12 probes at height C (1.75 m), and 3 probes at height D (2.5 m). For the control pile, a total of 4 probes were installed as controls – 1 probe installed at each height (0.5 m, 1.0 m, 1.75 m, and 2.5 m). The MITC monitoring network was less intensive than the pine-plug bioassay sampling, as it was intended to support evidence of fumigant presence and distribution, rather than a one-to-one match for all plug locations.

Figure 6. Intergranular MITC monitoring probes installed within the treated stockpile. Sampling lines were inserted through the slotted PVC tube infrastructure at predefined heights and lateral distances (Figure 7, Figure 8), with surface tubing connected to field gas-monitoring equipment for measurement of methyl isothiocyanate (MITC) concentrations within the gravel matrix.

3.4.4 Stockpile construction

Both stockpiles were constructed to a consistent crescent-shaped cross-sectional profile to support comparable sampling depths and lateral-distance classes between treated and control stockpiles (2.5 m depth, 3.5 m lateral distance). The treated stockpile consisted of three overlapping crescent-shaped stockpiles, while the control stockpile used the same general profile with one singular crescent. In accordance with MRWA's approved procedure, 1 m high bunds of pre-treated gravel were formed to surround both stockpiles (approximately 1 m × 1 m cross-section) as part of stockpile construction, to minimise segregation of larger material at the toe of the stockpile, which was identified as a high-risk zone for *Phytophthora* survival in 2019. Gravel moisture at the time of application was higher than average, and significant rainfall events were forecast in the days following the experiment and throughout the withholding period. This was considered sufficient for MITC generation and to form a surface crust without applying the standard water seal to the stockpile to slow atmospheric loss.

3.5 Application of metham sodium

3.5.1 Fumigation process overview

Metham sodium was applied to gravel as a water-based solution during stockpile construction. For the experimental treatment, metham sodium was mixed with water at the fumigation truck (positioned away

from the screen/conveyor) and pumped via approximately 121 m of 25 mm hose to the spray box mounted at the top of the stacker conveyor. Metham sodium is a Schedule 6 poison under the Australian Poisons Standard (SUSMP), and its degradant/active fumigant methyl isothiocyanate (MITC) is also Schedule 6; handling and use were therefore undertaken in accordance with the approved product label, Material Safety Data Sheet (MSDS), and Culford Quarry site safety controls.

Upon dilution, metham sodium hydrolyses to form MITC, the active gaseous fumigant. Fumigant performance and effective conversion to MITC may be influenced by experimental conditions, including dilution ratio, water chemistry (e.g., pH), and solution residence time prior to application. Pre-trial laboratory testing using site-sourced dam water confirmed rapid MITC formation under local water conditions, supporting the operational controls implemented in this trial.

Pre-trial gas chromatography–mass spectrometry (GCMS) testing confirmed that MITC forms rapidly after mixing and can volatilise quickly if exposed to air. Testing showed that MITC can be produced within minutes of mixing (with around 30% conversion by 3 minutes), and that once exposed to air, MITC can convert to gaseous MITC in under a minute (Nelson et al. 2013; Du and Dechen 2025). Accordingly, the delivery system and site layout were configured to reduce hose length and minimise atmospheric loss of MITC at the application point.

Once stockpiled, the rate of MITC loss from the gravel to the atmosphere is reduced, necessitating a minimum withholding period of at least 28 days before disturbance (per APVMA approved label). Independent MITC monitoring at the point of application, within the stockpile, and in the surrounding environment was undertaken by Murdoch University using ISO/IEC 17025-aligned procedures (Du et al. 2026).

3.5.2 Application rate, method and calibration

Intergranular monitoring of MITC during the 2024 trial showed a much lower cumulative exposure than expected by Murdoch University experts. Subsequent GCMS testing conducted prior to the present trial indicated that this was due to a significant loss of MITC at the spray box, due to conversion from the liquid metham sodium to the gaseous MITC commencing in the 121 m of hose between the mixing point at the truck and application at the spray box. Based on Murdoch expert advice and to compensate for measured losses prior to gravel contact, the APVMA approved dosage rate of 80 mL per m^3 of gravel was achieved by increasing the delivery rate at the mixing point to 173.1 mL/m^3.

Equipment was calibrated prior to construction of the treated stockpile by checking conveyor throughput and solution flow rate to the spray box. Conveyor throughput was then monitored throughout construction using a Loadrite® wheel-loader weigh scale to record tonnes loaded into the screen hopper at regular intervals, typically every 30 minutes. Tonnes were then converted to cubic metres using a gravel density of 1.667 t/m^3. Solution flow rate to the spray box was determined using the chemical tank weight scales/digital readout to determine kilograms of metham sodium pumped between readings, converting mass to volume using metham sodium density of 1.209 kg/L. Metham pumping rates were adjusted to achieve the target dose (mL/m^3). Metham sodium concentrate was diluted with water at a water: metham sodium ratio of 35.75:1, and the solution was continuously delivered to the spray box during stacking. Total volumes used during the treatment period were 13,104 L of water and 367 L of metham sodium.

3.6 Installation of inoculum and sampling infrastructure

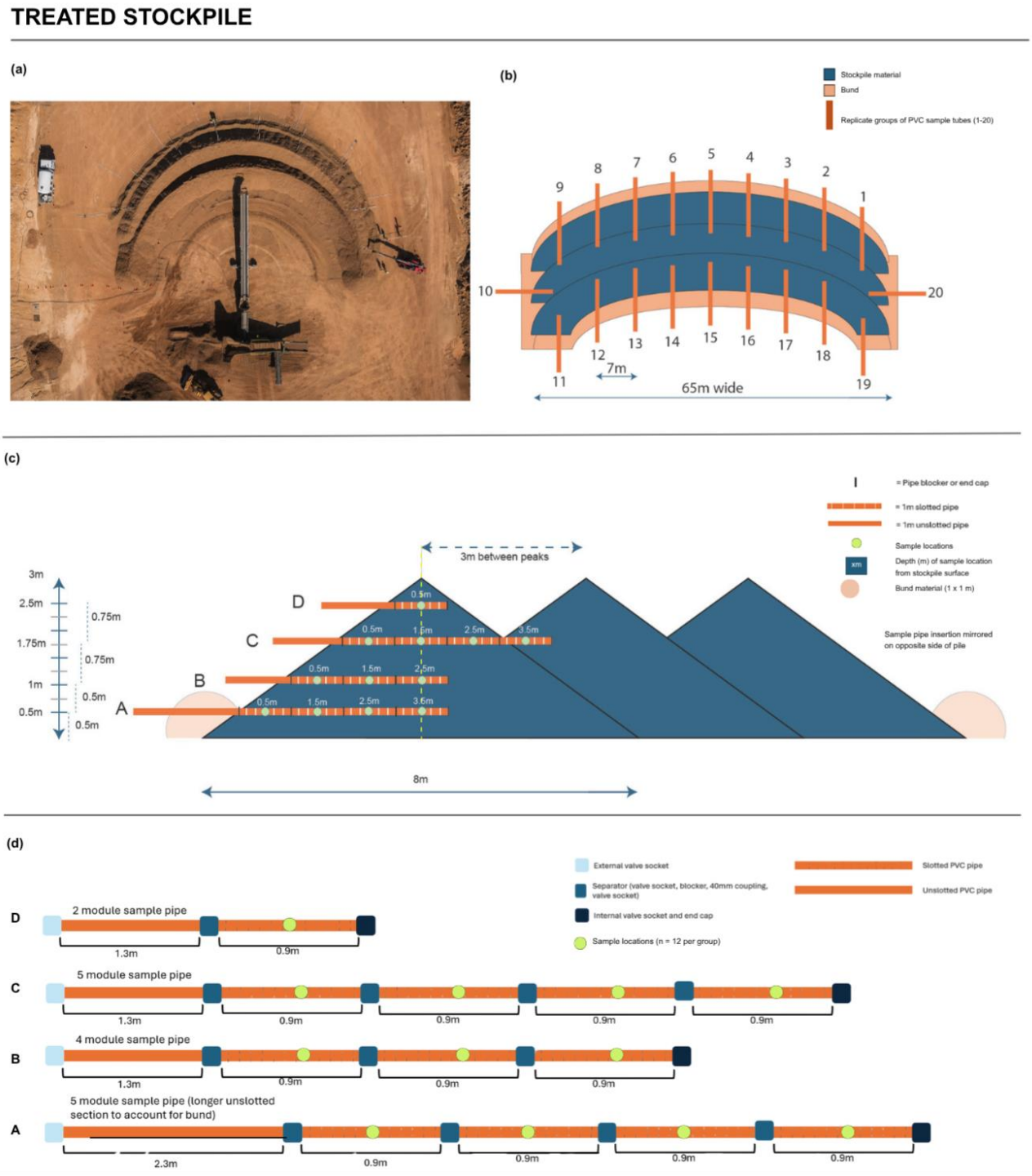


Figure 7. (a) Drone image of the metham sodium-treated stockpile for visual context and scale. The stockpile is approximately 65 m wide by 20 m long by 3 m high. The total volume of gravel used to construct the treated stockpile was 2,117.34 m³. (b) Top-down schematic of the treated stockpile, with lateral distribution of 20 groups of PVC pipes, installed every 7 m around the perimeter of the stockpile footprint. Each replicate group comprised four modular tubes, installed at four different heights, and contained a total of twelve sample locations (n = 240). (c) Cross-section schematic of treated stockpile, showing the vertical placement of the four PVC pipes within each replicate group (A = 0.5 m, B = 1.0 m, C = 1.75 m, and D = 2.5 m). Depths of sample locations relative to the surface of the stockpile are also depicted. (d) Diagram showing variations in lengths and number of samples for pipes A, B, C, and D, replicated across the 20 groups.

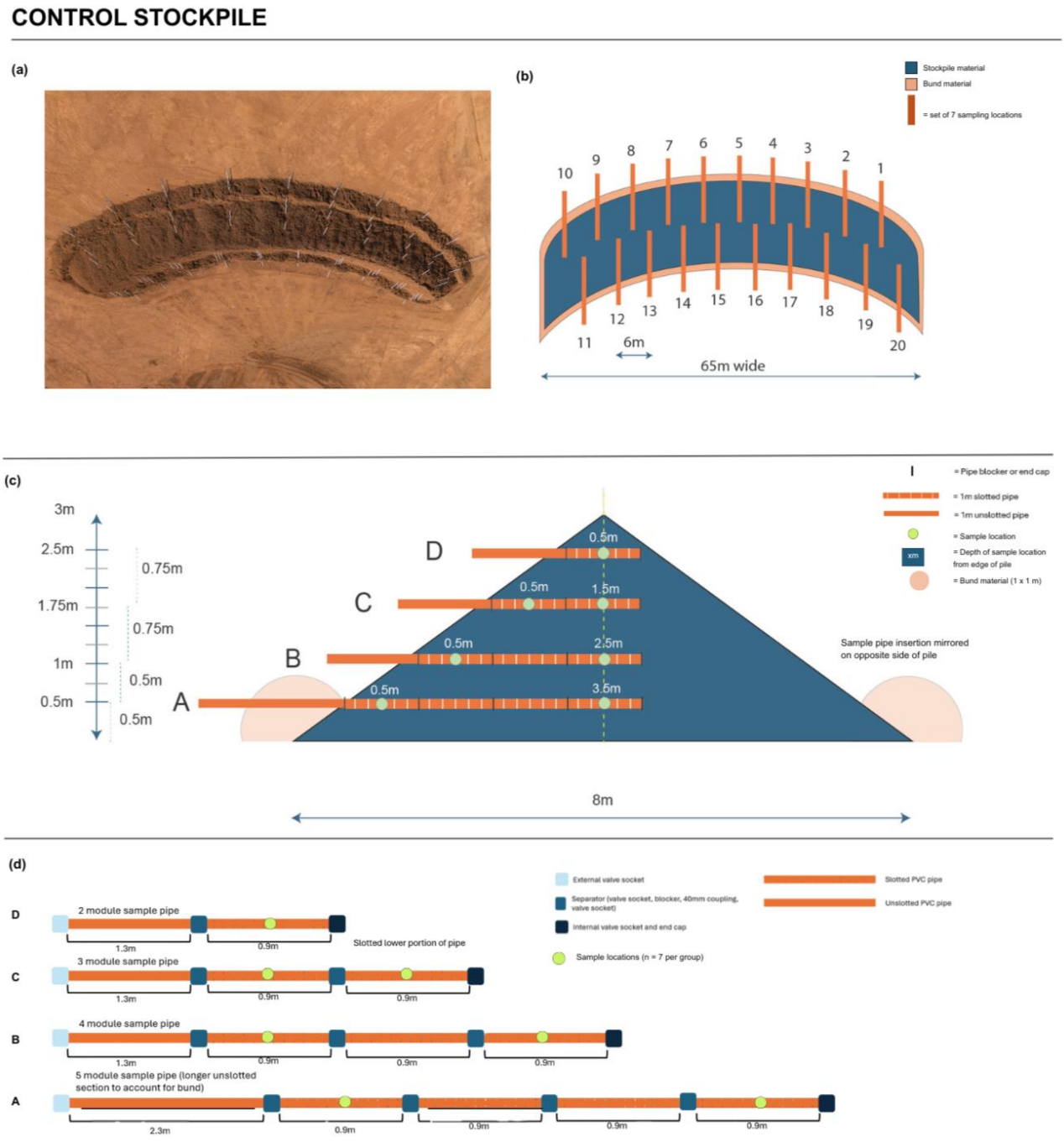


Figure 8. (a) Drone image of control stockpile for visual context and scale. The stockpile is approximately 65 m wide by 8 m long by 3 m high. The total volume of gravel used to construct the control stockpile was 528.91 m^3. (b) Top-down schematic of the control stockpile, with lateral distribution of 20 groups of PVC pipes, installed every 6 m around the perimeter of the stockpile footprint. Each replicate group comprised four modular tubes, installed at four different heights, and contained a total of seven sample locations (n = 140). (c) Cross-section schematic of control stockpile, showing the vertical placement of the four PVC pipes within each replicate group (A = 0.5 m, B = 1.0 m, C = 1.75 m, and D = 2.5 m). Depths of sample locations relative to the surface of the stockpile are also depicted. (d) Diagram showing variations in length and number of samples for pipes A, B, C, and D, replicated across the 20 groups within the control stockpile.

3.6.1 Installation method and traceability

Tubes were installed after stockpile construction was complete using a Manitou telehandler fitted with a 6 m steel prong, suitable for maximum pipe lengths of approximately 5.5 m and a prong diameter of ~100 mm (Figure 9). The prong was inserted into the stockpile at the predefined insertion points and withdrawn to create pilot holes. PVC tubes were then immediately inserted manually into the pilot holes by hand. Tubes

were colour coded with spray paint to match the Burley cages to maintain traceability of the different *Phytophthora* isolates/species through retrieval and laboratory processing (Figure 2).

Figure 9. Manitou 1840 telehandler fitted with steel-prong used to install monitoring tubes into the gravel stockpiles during the trial.

3.7 Inoculum plug retrieval and *Phytophthora* retrieval

3.7.1 Extraction of samples

Following completion of the fumigation exposure (withholding) period (59 days, from 4 September to 3 November 2025), samples were retrieved from both the treated and control gravel stockpiles. The withholding period was extended beyond the anticipated 28 days to allow additional time for MITC levels to reach an acceptable level, for occupational health and safety reasons. Retrieval was undertaken by locating the exposed ends of the sampling tubes and carefully withdrawing the modular PVC sampling assemblies from the stockpile. Burley cages containing the inoculated pine plugs were removed immediately after extraction and kept segregated by stockpile and sampling location (Figure 7, Figure 8) and packed into sterilised boxes for transport to VHS.

3.7.2 Sample handling and transport

Each recovered Burley cage (two plugs per cage) was individually labelled using the trial sample ID system and sealed in clean containers to remove the risk of cross-contamination and moisture loss during handling. Samples were transported to the VHS laboratory for viability testing. Transport occurred on the same day after extraction, with samples stored within an insulated container out of direct sunlight.

3.7.3 Outcome definitions

Inoculated pine plugs were initially processed through direct plating, with a follow-up baiting procedure only conducted on the negative samples to confirm the absence of *Phytophthora.* The purpose of this approach

was to reduce false negatives by combining two complementary recovery methods while applying a clear, conservative rule for treatment-efficacy assessment.

- Positive (non-sterilised): A sampling location was recorded as positive if the *Phytophthora* species was recovered by either method (direct plating or baiting) from that same location (i.e., any recovery event counted as a positive outcome).
- Negative (sterilised): A sampling location was recorded as negative if *Phytophthora* was not recovered by either method from that location after completion of the assessment period(s).
- Compromised (NA result): Samples were compromised, and the location was removed from the dataset for analysis.

3.7.4 Assessment of Phytophthora survival

Survival of *P. cinnamomi* and *P. multivora* in samples was assessed by the VHS, using standard *Phytophthora* diagnostic methods consistent with previous metham sodium gravel trials (Davison et al. 2019). Inoculum recovery was assessed using two complementary pathways: (i) direct plating of processed pine plug material onto *Phytophthora*-selective agar, and (ii) baiting-based recovery from wood samples (19 November 2025). Morphological identification was used to confirm that *P. cinnamomi* and *P. multivora* were the species present after baiting and plating were complete.

Direct plating: The samples were surface-sterilised (70% ethanol for 30 seconds; rinsed twice in sterile de-ionised water), dried, split longitudinally, and plated onto *Phytophthora*-selective agar following the approach used in Davison et al. (2019). Plates were incubated at ambient temperature (~22 °C) and monitored for up to 14 days for *Phytophthora* growth (Davison et al. 2019).

Baiting: Samples were processed for baiting by VHS following their standard diagnostic workflow. Outcomes were recorded at the sample-location level and used alongside plating results to determine the final viability for each location. No molecular identification was undertaken. Positive recoveries were determined using morphological confirmation consistent with VHS standard practice and the 2019 trial approach. To optimise laboratory effort and focus confirmatory testing on potential false negatives, baiting was applied only to samples that were initially scored as negative by direct plating. Samples with *Phytophthora* recovery via direct plating were already confirmed positive (i.e., non-sterilised) and were therefore not subjected to additional baiting.

4 Data handling and statistical analyses

The trial comprised one metham sodium-treated stockpile and one untreated control stockpile, so treatment effects are interpreted as a comparison between stockpiles under the tested operational conditions. Sampling depth and lateral distance were included as spatial covariates to assess within-stockpile variation and support interpretation of treatment performance across the sampled profile. The control stockpile was smaller than the treated stockpile and contained fewer sampling locations; however, sampling depths, lateral-distance classes, and installation methods were comparable between stockpiles. In the case that no result was available or could not be validated, those data points were excluded from percentage calculations and from statistical modelling (NA).

4.1 Intergranular MITC and sterilisation

Intergranular MITC monitoring data were modelled against positive locations to explore whether fumigant levels inside the stockpile were associated with sterilisation outcomes at nearby plug locations. MITC was measured over time at fixed gas-sampling points, and for each point we calculated a cumulative exposure value (C×T, mg·h/L) and simple summary statistics of MITC concentration (mean and maximum, ppm).

For this analysis, results include only locations in the metham sodium-treated stockpile that had both determinate plug results and an associated MITC monitoring point. Plug outcomes were then aggregated to the location level: for each treated location, we counted the number of plugs tested, and the amount that were sterilised or remained positive for *Phytophthora*.

A binomial generalised linear model (logistic regression with logit link) was fitted to the treated locations, using location-level sterilisation counts (sterilised vs recovered) as the response. Cumulative MITC exposure (C×T; mg·h/L) was the main predictor, scaled by 1,000 (C×T/1000, mg·h/L) so that model coefficients described the change in odds of sterilisation per 1,000 mg·h/L increase in MITC exposure.

Model stability was assessed because only a small number of locations showed *Phytophthora* survival, and overall sterilisation rates were high. Influence diagnostics (leverage and Cook's distance) were examined to check that model estimates were not driven by single locations. As a sensitivity check for small-sample bias and potential separation, a bias-reduced (Firth) logistic regression, with the same response and predictor structure, was also fitted using the logistf package. The results were qualitatively compared with those of the standard binomial GLM. Given the high sterilisation rate and sparse survival outcomes, these analyses were treated as exploratory and interpreted primarily in terms of effect direction, uncertainty (95% confidence intervals), and robustness across the standard and Firth models.

4.2 Primary treatment efficacy model

Treatment efficacy was assessed using a binomial generalised linear model (logistic regression) with treatment (treated vs control) as the predictor. Results are reported as odds ratios (OR) with 95% confidence intervals. Model checks included assessing influential observations and outcome separation; when separation was detected, a bias-reduced logistic regression approach was used to produce stable estimates.

4.3 Adjusted model including spatial and biological covariates

To test whether the treatment effect remained after accounting for other factors, an adjusted binomial logistic regression was fitted, including treatment, species, sampling depth, and lateral distance. Isolate was

not included in the main adjusted model because isolate identity was species-specific, rendering isolate and species estimates non-independent and leading to unstable coefficient estimates when both were included.

4.4 Sensitivity to within-location non-independence

Multiple plug outcomes were associated with the same sampling location, and the number of unique locations was limited (12 locations). We therefore assessed sensitivity to potential within-location non-independence. In addition to the primary adjusted binomial logistic regression (treatment + species + depth + lateral distance), we refitted the same model and calculated small-sample cluster-robust (CR2) standard errors and 95% confidence intervals clustered by location. Cluster-robust results were treated as a sensitivity check on inference (SEs/CIs/p-values) rather than a change to point estimates (odds ratios), and interpretation focused on whether conclusions about treatment and species effects were consistent under this more conservative error structure.

5 Results

Sterilisation of gravel using metham sodium has the potential to enhance current management strategies for mitigating dieback spread. To evaluate this approach, we monitored the presence of *P. cinnamomi* and *P. multivora* in the treated gravel stockpile, alongside spatial and temporal variation and intergranular MITC concentrations. Of the 240 cage-matched sample locations in the treated stockpile, 2 cages (1 cage contained *P. cinnamomi* isolate 9701, and 1 cage contained *P. cinnamomi* isolate 37439) were compromised during removal, resulting in 238 locations for statistical analysis. All remaining samples were retrieved successfully from the 140 control locations. A visual summary of the results by outcome and distributions is presented in Figure 10.

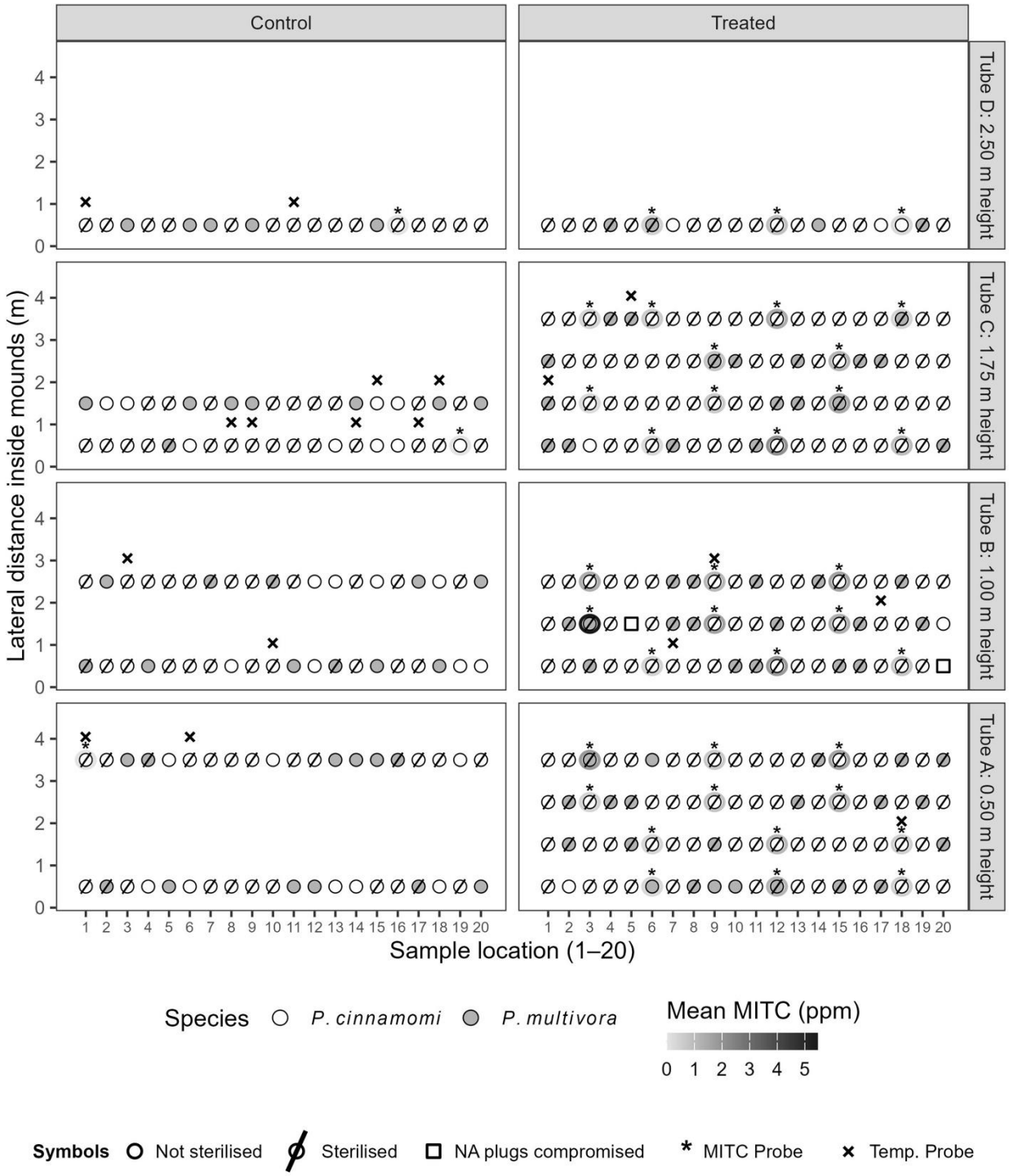


Figure 10. Spatial outcomes of Phytophthora *sample results by group (1-20; x-axis), and lateral distance into the stockpile (y-axis). Panels compare the untreated control (left) and metham sodium-treated (right) stockpiles, arranged by sampling height above ground (0.50, 1.00, 1.75 and 2.50 m). The locations of intergranular MITC monitoring points and temperature data loggers are also depicted.*

5.1 Efficacy of metham sodium treatment

Statistical analyses were conducted by sample location (cage), where each location comprised two pine plugs (four plated halves). A sample was classified as negative only when no recovery occurred in all subsamples (by either direct plating or baiting).

Phytophthora survival across both species and isolates was significantly lower in the treated material, with 227 of 238 (95.4%) testing negative for *Phytophthora* (Table 5), compared with 88 of 140 (62.9%) control locations. This difference was strongly supported by a binomial logistic regression, with treated samples having higher odds of sterilisation than controls (odds ratio = 12.19, 95% CI 6.30–25.63; $p < 0.001$).

Analysis of metham sodium treatment efficacy by *Phytophthora* species was key, as the emphasis for *Phytophthora* dieback management in WA is placed on *P. cinnamomi*, both from a policy and impact perspective. Sterilisation outcomes varied between *Phytophthora* species (adjusted species effect: *P. multivora* vs *P. cinnamomi*, OR = 0.155, 95% CI 0.075–0.310; $p < 0.001$; Table 8). In treated samples, high levels of sterilisation were observed for both *P. cinnamomi* (171 of 177 samples tested; 96.6%) and *P. multivora* (56 of 61; 91.8%). In contrast, control samples showed lower sterilisation rates, particularly for *P. multivora* (9 of 36; 25%), while the *P. cinnamomi* control samples also had a high negative rate (79 of 104; 76.0%).

Analysis by isolate was also performed, with descriptive isolate-level outcomes summarised in Table 5. In the treated stockpile, sterilisation rates were markedly higher across all isolates, with only 1 to 5 sample locations showing recovery per isolate group. The highest sterilisation rate within the treated pile was observed for *P. cinnamomi* isolate 9701, with 59 of 60 sample locations sterilised (98.3%), while *P. cinnamomi* isolate 37439 showed a similarly high sterilisation rate of 57 of 58 sample locations (98.3%). *Phytophthora multivora* isolate 37571 had the highest recovery rate among treated isolates, with 56 of 61 sample locations sterilised (91.8%). In the control stockpile, negative sample locations ranged from 25% for *P. multivora* isolate 37571 to 85.3% for *P. cinnamomi* isolate 9701. This variation reflects substantial background variability between isolates in the absence of fumigation. The results demonstrate consistent isolate-level efficacy of metham sodium treatment and support modelling treatment effects at the species level.

Table 5. Negative recovery outcomes by metham sodium treatment and Phytophthora *isolate. A negative sample location was defined as one where no* Phytophthora *was recovered from any plated or baited subsample. Percentages are based on tested sample locations only, excluding unavailable or indeterminate outcomes. Results are descriptive; isolate identity was not included as a separate term in the regression models. Two treated sample locations were unavailable, one for isolate 9701 and one for isolate 48828. Sample-location numbers per isolate were not identical due to randomised isolate placement and the exclusion of unavailable locations.*

Stockpile	Species	Isolate code	Negative sample locations by isolate (%)	Negative sample locations by species (%)	Negative sample locations all (%)
Control (n=140)	*P. cinnamomi* (n=104)	*9701* (n=34)	85.3% (29 of 34)	76% (79 of 104)	62.9% (88 of 140)
		37439 (n=35)	82.9% (29 of 35)		
		48828 (n=35)	60.0% (21 of 35)		
	P. multivora (n=36)	*37571* (n=36)	25.0% (9 of 36)	25.0% (9 of 36)	
Treated (n=238)	*P. cinnamomi* (n=177)	*9701* (n=60)	98.3% (59 of 60)	171 / 177 (96.6%)	95.4% (227 of 238)
		37439 (n=58)	98.3% (57 of 58)		
		48828 (n=59)	93.2% (55 of 59)		
	P. multivora (n=61)	*37571* (n=61)	91.8% (56 of 61)	56 / 61 (91.8%)	

5.2 MITC monitoring

Du et al. (2026) quantified MITC concentrations during and after metham sodium application using three complementary monitoring objectives: (i) spray box monitoring to quantify loss rates and confirm that adjusted dosage is consistent with APVMA advice; (ii) intergranular monitoring to describe the movement of MITC through the stockpile; and (iii) perimeter monitoring to further inform current safety protocols on the size of the exclusion zone and buffer (currently a 200 m exclusion zone is required by the APVMA). Results are summarised below.

5.2.1 Vertical monitoring at the spray box

Monitoring points were installed in vertical alignment at the spray box to compare MITC concentrations at an upper and lower position. Spray-box monitoring showed consistently higher MITC concentrations at the lower position (mean 1.11 ppm) than at the upper position (mean 0.41 ppm), with concentrations peaking at hour 4 (2.03 ppm lower; 1.01 ppm upper).

5.2.2 Intergranular monitoring and cumulative exposure

Intergranular monitoring within the treated stockpile used fixed gas-sampling points to extract samples over time to define cumulative exposure (expressed as C×T, mg·h/L): Intergranular monitoring within the treated stockpile (n=36 sampling points) yielded C×T values ranging from 512.7 to 8,122.8 mg·h/L (mean = 2,219.7 mg·h/L). MITC exposure (C×T; mg·h/L) was variable throughout the stockpile (Figure 11) both in relation to height above ground level (Figure 11a) and depth from the surface of the stockpile (Figure 11b). Based on results, Du et al. (2026) defined an indicative C×T threshold of 700 mg·h/L required to effectively eliminate *Phytophthora* from gravel. While most locations exceeded the proposed exposure threshold, four points below 700 mg·h/L, with two linked to recovery, were identified and seemed to be associated with *Phytophthora* recovery (Table 6), indicating a relationship between low MITC exposure and treatment efficiency (*Phytophthora* elimination).

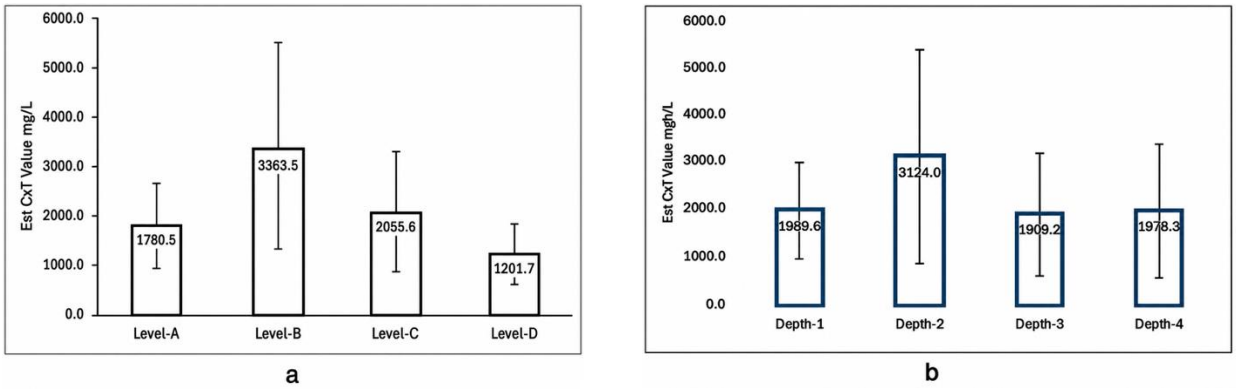


Figure 11. Cumulative MITC exposure values (C×T; mg·h/L) by (a) sampling height and (b) lateral depth within the treated gravel stockpile. Error bars represent ±1 standard deviation. Heights/levels A, B, C and D correspond with the convention used for bioassay sample locations. Source: Du et al. (2026).

5.2.3 Perimeter monitoring

Environmental perimeter monitoring detected MITC above the analytical limit of detection (LoD; 0.02 ppm) primarily within the first two weeks post-application at sampling points located 1, 5, 10, 50, 100, 150 and 200 m from the point of application along east, west, north and south transects. The highest off-stockpile concentration recorded was 2.18 ppm at 50 m east. The most persistent detections occurred downwind to the west, extending to day 31 post-application.

Table 6. Summary of intergranular (CxT) and perimeter MITC monitoring outcomes reported by Du et al. (2026), and locations associated with low exposure and plug recoveries.

Monitoring component	Metric	Summary result
Spray-box vertical monitoring	Mean MITC (upper / lower)	0.41 ppm / 1.11 ppm
Spray-box vertical monitoring	Peak MITC (upper / lower) and timing	1.01 ppm / 2.03 ppm (hour 4)
Intergranular monitoring (treated stockpile)	Number of sampling points	36 points
Intergranular monitoring (treated stockpile)	C×T (mg·h/L), mean (range)	2,219.7 (512.7–8122.8) mg h/L
Intergranular monitoring (treated stockpile)	Points below 700 mg·h/L threshold	4 points: T-3C3, T-6A4, T-6C4 and T-18D1
Environmental air monitoring ("perimeter")	Monitoring layout	Four directions (N, W, S, E) at 1, 5, 10, 50, 100, 150 and 200 m from the point of application
Low-exposure points linked to plug recoveries	Location codes	T-6A4 and T-18D1
Environmental (perimeter) monitoring	Detection limit	0.02 ppm
Environmental (perimeter) monitoring	Maximum detected concentration	2.18 ppm

5.3 Spatial distribution effects for bioassay outcomes

Sterilisation rates within the treated stockpile were high throughout its profile. Statistical analysis found no conclusive relationship between spatial location and *Phytophthora* survival (Table 7). However, a weak observational pattern may indicate a tendency for positive recoveries to be concentrated at near-edge locations, particularly at 0.5 m lateral distance, where 9 of the 11 treated-pile recoveries occurred. Most of the deeper into-pile positions were fully sterilised, although isolated recoveries occurred at A–3.5 m and B–1.5 m (Table 7). Where MITC monitoring overlapped with recovery locations (Figure 10), the two monitored recovery locations, T-6A4 and T-18D1, had intergranular C×T values below the proposed exposure threshold of approximately 700 mg·h/L defined by Du et al. (2026).

Table 7. Sterilisation outcomes within the treated stockpile by pipe level and lateral distance into the stockpile. Values show the percentage and count of tested sample locations with no Phytophthora *recovery.*

	Sample depths			
Pipe level	0.5 m	1.5 m	2.5 m	3.5 m
A	80% (16/20)	100% (20/20)	100% (20/20)	95% (19/20)
B	100% (19/19)	94.7% (18/19)	100% (20/20)	NA
C	95% (19/20)	100% (20/20)	100% (20/20)	100% (20/20)
D	80% (16/20)	NA	NA	NA

5.3.1 Adjusted sterilisation model (treatment, species and spatial covariates)

An adjusted binomial logistic regression including treatment, *Phytophthora* species, sampling depth, and lateral distance confirmed a strong treatment effect on sterilisation outcomes (Table 8). Isolates were treated as biological replicates nested within species, as modelling isolate identity separately would introduce additional parameters relative to the small number of positive outcomes and reduce model

stability. Species was retained as the biologically meaningful grouping for inference. After adjustment, treated samples had higher odds of sterilisation than control samples (OR = 15.9, 95% CI 7.641 - 36.266; p < 0.001). Species identity also influenced outcomes, with *P. multivora* showing lower odds of sterilisation than *P. cinnamomi* (OR = 0.155, 95% CI 0.075–0.31; p < 0.001). There was no strong statistical evidence that sterilisation probability varied with sampling height within the tested range (p > 0.05). Sterilisation showed a weak tendency to increase with distance into the pile from the stockpile edge (odds increased by ~29% per additional metre; OR = 1.29; p = 0.12). Lateral distance was modelled as a continuous covariate (per metre); treating distance as categorical did not improve model fit (likelihood ratio test: Deviance = 0.68 on 2 df; p = 0.71).

Table 8. Adjusted binomial logistic regression results for sterilisation outcomes across all tested sampling locations in the control and treated stockpiles. Sterilisation was defined as recovery of no Phytophthora *("neg") at a sampling location versus any recovery. Predictors were metham sodium treatment, species (*P. multivora *vs* P. cinnamomi*), sampling height above ground, and lateral distance into the pile from the stockpile edge. Sampling height was modelled with 0.5 m as the reference level, and lateral distance was modelled per metre. Results are presented as odds ratios (OR) with 95% confidence intervals (CI) and p-values.*

Predictor	Odds ratio (OR)	95% CI	p-value
Metham sodium treatment (treated vs control)	15.888	7.641-36.264	<0.001
Species (*P. multivora* vs *P. cinnamomi*)	0.155	0.075-0.310	<0.001
Height above ground: 1 m (vs 0.5 m)	1.563	0.667-3.756	0.309
Height above ground: 1.75 m (vs 0.5 m)	1.396	0.597-3.306	0.443
Height above ground: 2.5 m (vs 0.5 m)	1.368	0.448-4.366	0.587
Lateral distance (per 1 m)	1.289	0.940-1.789	0.120

5.3.2 Sensitivity analysis: clustering by sampling location

As a sensitivity check for potential non-independence within sampling locations, we refit the adjusted model using small-sample cluster-robust (CR2) standard errors clustered by sampling location. The primary conclusions were unchanged. The treatment effect remained strong (OR = 15.89; CR2 95% CI 3.51–71.82; p = 0.002), and species identity remained an important predictor (*P. multivora* vs *P. cinnamomi*: OR = 0.155; CR2 95% CI 0.054–0.450; p = 0.004). The lateral distance effect remained comparatively uncertain (OR = 1.29 per metre; CR2 95% CI 0.806–2.062; p = 0.170), supporting cautious interpretation of spatial trends relative to the consistent treatment and species effects.

5.3.3 Localised intergranular MITC exposure and spatial effects on sterilisation

Intergranular MITC exposure was evaluated opportunistically as a potential explanatory variable for location-level bioassay outcomes at treated locations matched to MITC monitoring points (n = 36). For each monitoring point, MITC was measured over time at fixed gas-sampling locations and summarised as cumulative exposure (C×T, mg·h/L) and simple concentration statistics (mean and maximum, ppm). These monitoring-point summaries were matched to plug locations using a shared location identifier, and plug outcomes were summarised at the location level (sterilised vs any recovery). Across the 36 treated monitoring points, cumulative MITC exposure (C×T) ranged from 512.7 to 8,122.8 mg·h/L (mean 2,219.7 mg·h/L, median 2,029.2 mg·h/L), indicating substantial spatial variation in in-pile MITC exposure. Only two of the 36 MITC monitoring points matched locations where *Phytophthora* was recovered from the plugs (Figure 10).

Four monitoring points across the treated stockpile showed MITC rates to be below the indicative 700 mg·h/L threshold for successful treatment. Two of these four points (T-6A4 and T-18D1) corresponded to locations

where *Phytophthora* was recovered. This result suggests an association between low C×T values and reduced treatment efficacy, but the small number of recovery events means this relationship should be interpreted cautiously. In a treated-location binomial logistic regression (logit link), cumulative MITC exposure (C×T, scaled per 1,000 mg·h/L) was not statistically significant using the standard Wald test ($p = 0.245$), with wide uncertainty, reflecting the small number of recovery events and limited power. As a sensitivity check for small-sample bias and potential (quasi-)separation, a bias-reduced (Firth) logistic regression was also fitted. The Firth model provided weak evidence for an exposure effect based on the profile penalised-likelihood test (OR = 7.51 per 1,000 mg·h/L; $p = 0.081$), but confidence limits were extremely wide, and convergence warnings were produced. Overall, this analysis assessed whether localised MITC exposure corresponded with the observed pattern of *Phytophthora* recovery in the treated stockpile. The results support a plausible association between low C×T values and recovery at some locations, but the small number of positive outcomes means this should be interpreted as exploratory rather than as a definitive dose–response relationship.

5.4 Intra-stockpile temperature ranges

Temperature probes were deployed at 12 locations in the untreated control stockpile and 6 locations in the metham sodium-treated stockpile, all coinciding with plug bioassay locations (Figure 10, Table 9). Additional temperature probes were installed in the control stockpile following the 2024 trial, in which unexpectedly high inoculum mortality was recorded, to explore temperature as a covariate contributing to plug mortality that might otherwise have been attributed to metham sodium. Extreme intra-stockpile temperatures outside the optimum growth range of *P. cinnamomi* (12 to 35 °C) would have been flagged as a potential experimental issue because they could contribute to plug mortality independently of metham sodium exposure. No sustained high-temperature conditions likely to cause heat-related mortality were recorded.

Across the probe-matched locations, mean internal temperatures over the monitoring window ranged from 13.8–16.3 °C in the control stockpile and 15.0–16.6 °C in the treated stockpile (Table 9), well below temperatures expected to cause heat-related mortality. Minimum temperatures ranged from 7.6–11.9 °C (control) and 10.2–12.9 °C (treated), while maximum temperatures ranged from 16.8–22.9 °C (control) and 17.4–19.6 °C (treated).

Table 9. Probe-level summary statistics for internal control and treated stockpile temperatures (°C), collected over the common analysis window (5 September 2025 08:00 to 3 November 2025 08:00 AWST). Values are summarised by probe location (Figure 10).

Stockpile	Probe.ID	Number of logs (n)	Mean (°C)	Standard Deviation (±)	Minimum (°C)	Maximum (°C)
Control	1 A 1	16992	14.4	1.6	11.9	16.8
Control	1 D 1	16992	15.8	3.0	7.6	22.9
Control	3 B 1	16992	15.4	2.0	12.0	18.5
Control	6 A 1	16992	14.2	1.9	10.7	16.9
Control	8 C 2	16992	14.0	2.2	10.2	17.2
Control	9 C 2	16992	14.5	2.2	10.6	17.7
Control	10 B 2	16992	15.0	2.1	11.0	18.1
Control	11 D 1	16992	16.3	2.7	11.0	20.8
Control	14 C 2	16993	16.3	2.7	10.9	21.2
Control	15 C 1	16992	15.0	2.6	10.6	19.1
Control	17 C 2	16992	13.8	2.4	9.5	17.2
Control	18 C 1	16992	14.9	2.2	11.1	18.6
Treated	1 C 3	16992	15.2	1.7	12.5	17.7
Treated	7 B 3	16992	15.0	2.1	10.2	18.8
Treated	9 B 1	16992	15.1	1.6	12.7	17.4
Treated	15 C 1	16992	15.5	2.3	10.4	19.3
Treated	17 B 2	16992	16.0	2.2	12.4	19.5
Treated	18 A 3	16992	16.6	2.0	12.9	19.6

6 Discussion

This trial provides strong evidence that metham sodium treatment can reduce the probability of detectable *Phytophthora* survival within gravel under commercial stockpile conditions, specifically for *P. cinnamomi* and *P. multivora*. Statistical analyses demonstrated significantly higher sterilisation rates in treated material compared with the untreated control stockpile, although the magnitude of the treatment effect varied between species and, to a lesser extent, isolates. No statistically significant spatial patterns were detected in the distribution of *Phytophthora*-positive samples within either stockpile. While several positive detections occurred in areas of comparatively low MITC exposure (< ~700 mg·h/L), the number of positive detections was too small to support robust statistical inference regarding the response of isolates to variable MITC exposures. Nevertheless, these findings suggest that localised low-exposure zones within the stockpile may contribute to treatment failure under some operational conditions and provide a useful basis for further optimisation of fumigant delivery systems.

A key objective of this project was to assess whether operational refinements implemented following the 2019 trial (Williams and Eslick 2019) improved treatment performance under commercial-scale conditions. In the present trial, 227 of 238 treated sample locations were negative for *Phytophthora* recovery, representing an overall negative recovery rate of 95.4%. This result was numerically higher than the 94.2% negative recovery rate reported by Williams and Eslick (2019), suggesting that the revised treatment and stockpile management procedures maintained, and may have modestly improved treatment performance. However, the difference in negative recovery rates between trials (94.2% and 95.4%) was small and should be interpreted cautiously because the trials were not designed as direct paired comparisons and differed in several operational and environmental variables.

Importantly, outcomes varied between species, with negative recovery observed at 171 of 177 *P. cinnamomi* locations (96.6%) and 56 of 61 *P. multivora* locations (91.8%). The distinction is important for environmental managers and policy-makers when assessing the suitability of metham sodium-treated gravel for different use cases, particularly because *P. cinnamomi* is often the primary management priority under both legislative frameworks relating to EPBC Act Key Threatening Processes and broader Phytophthora dieback management programs (Department of Biodiversity, Conservation and Attractions 2023).

The bioassay design used in this trial was intentionally conservative and developed to provide a rigorous operational stress test of treatment efficacy. The inoculated pine plugs represented concentrated, protected *Phytophthora* inoculum sources housed within perforated Burley cages, rather than naturally dispersed inoculum within gravel matrices. The outcomes presented here should not be interpreted as direct estimates of survival probability in naturally infested gravel under field conditions. Instead, the trial provides a controlled comparative assessment of treatment performance under defined operational conditions using a highly standardised experimental system.

Within the stockpile, no statistically significant spatial patterns were identified in the distribution of sterilised versus positive plugs. This is a noticeable change from the 2019 research, where positive retrievals were concentrated in an area of concern at the toe of the stockpile, where material was likely to segregate, increasing the evaporation rate of MITC, resulting in lowered cumulative exposure. The absence of a comparable spatial effect in the present study suggests that the revised stockpile design, including installation of perimeter bunds, improved MITC retention and reduced the segregation-related treatment variability observed in 2019.

These results demonstrate that metham sodium treatment reduces the likelihood of detecting *Phytophthora* (specifically *P. cinnamomi* and *P. multivora*) in treated BRM. Replication of the 2019 field trial with targeted refinements produced recovery and negative rates that were broadly consistent with previous findings, corroborating the high efficacy reported earlier and strengthening confidence in performance under real-world implementation. From an operational perspective, these findings indicate that when stockpiles are constructed and treated in accordance with current protocols and specifications, metham sodium fumigation can serve as an effective risk-mitigation tool for producing BRM with reduced Phytophthora dieback risk.

Across the monitoring period, recorded temperatures were within the understood temperature range for *P. cinnamomi* growth (12–35 °C), with 25 °C considered the optimum. All results were below the reported maximum growth temperature for *P. multivora* of approximately 32.5 °C (Erwin and Ribero 1996), supporting the interpretation that the negative recovery outcomes observed in treated samples were attributable to metham sodium/MITC exposure rather than heat-related mortality.

Addressing residual risk – interpreting positive sample outcomes

Few samples remained positive for *Phytophthora* after the metham sodium treatment, representing a 3.4% residual risk for *P. cinnamomi* (6 of 177) and an 8.2% residual risk for *P. multivora* (5 of 61). Until further data is collected, metham sodium fumigation should be viewed as a risk-mitigation treatment rather than a guaranteed sterilisation approach. In practice, this treatment should be applied as part of an integrated management framework that also includes site selection and hygiene controls, sourcing from low-risk quarries where possible, and appropriate post-treatment surveillance. As detection technologies continue to improve (e.g., more sensitive molecular diagnostics (Wilkins et al. 2025), field-deployable assays (Mainello-Land et al. 2025), and detection dogs), there is an opportunity to pair metham sodium fumigation with more rapid verification of *Phytophthora* status, strengthening confidence in treated material.

Practical constraints and the conservative role of the plug bioassay

Directly confirming the absence of *Phytophthora* throughout a large industrial stockpile by destructive sampling is not feasible, as the volume of gravel required for testing would be logistically prohibitive and would compromise the operational value of the material. The plug bioassay using Burley cages in slotted PVC tubes, therefore, remains a practical, deliberately conservative way to assess treatment performance at scale, which has been used since the initial 2012 field trial. Through this design, samples are in fixed, known locations within the pile and any recovery of *Phytophthora* at a location is counted as a failure.

There are some practical limitations to this approach, which may influence the results. Field observations suggest that the slotted PVC tube-and-cage microenvironment may include zones less accessible to gas than the surrounding gravel. This is because gravel fines combined with moisture may form a film or compacted area impeding MITC movement into parts of the sampling system (Figure 12). Under these conditions, positive detections may reflect micro-scale barriers introduced by the sampling design rather than treatment failure. As a result, the assay is likely biased toward over-detecting residual *Phytophthora* risk rather than under-detecting it. This interpretation is also consistent with observations linking lower MITC exposure to reduced sterilisation rates; however, it cannot be directly verified with the available data.

The very high proportion of negative locations in the treated stockpile, supported by the MITC monitoring data, provides strong evidence that the protocol delivers high treatment efficacy and substantial risk mitigation, while recognising that any sample-based bioassay cannot eliminate residual uncertainty. The PVC

pipe and bioassay methodology remain appropriate for testing the efficacy of the fumigation method, recognising that practical limitations may inflate residual risk metrics.

Figure 12. Close-up of a slotted PVC sample pipe immediately after extraction from the stockpile.

Policy support, practical implementation and monitoring

Given the public investment to date and the known limitations of the plug-bioassay methodology, it is considered that future large-scale trials of this nature may yield diminishing returns in resolving the remaining sources of residual uncertainty. It is recommended that the treatment approach now be considered for broader operational implementation under specific conditions, with respect to land tenure and an acceptable level of risk to adjacent ecological assets. Information on this product, including procurement guides, information on its strengths and limitations, and how to assess the suitability of a site for product use, should be incorporated into state and community-owned best practice guides and manuals, with the potential to introduce a Certification system for metham sodium-treated gravel for ease of decision-making for land managers.

With respect to land tenure, a clear policy stance is needed for lands managed under the Conservation and Land Management Act. Leadership for this must come from WA State Government (DBCA), and development of a risk-dependent decision matrix for use on a site-by-site basis has been requested through DBCA's Conservation and Environmental Management Division, which oversees plant disease-related policy within the Department. For other land tenures, such as Local Government Reserves and Unallocated Crown Land, this product should now be considered as a commercially available lower disease risk alternative to other common gravel sources, such as that sourced from farm paddocks (Main Roads Western Australia, 2023). The DWG, DBCA, and MRWA subject matter experts are available for consultation support on the appropriate use of this material within the context of the proposed use case.

As a key part of the recommended implementation pathway, annual Phytophthora dieback monitoring of sites where metham sodium-treated gravel has been used in civil construction activities is strongly encouraged as it will provide longitudinal, real-world data insights into addressing residual risk. If disease expression potentially associated with gravel movement is observed, DBCA's Disease Hygiene and Standards

Officer should be contacted immediately for an assessment of the site (including soil sampling), and an expert opinion on the likely vector for disease expression, gravel or otherwise.

6.1 Research and Development Opportunities

Alongside operational implementation, the following research and development opportunities were identified to further optimise MITC delivery and treatment consistency within gravel stockpiles:

1. Investigate the potential to develop a certification scheme for 501 Specification gravel, which has been treated with metham sodium.

   (a) The lack of a recognised and auditable certification framework for treated gravel that is Phytophthora-free or with reduced Phytophthora risk remains a practical challenge for land managers, particularly in procurement processes, contractor specifications, and internal operational procedures. Stakeholders from industry, state government, local government, and environmental management sectors should therefore be included in any scoping and design phase assessing the feasibility and utility of a certification system.

2. One practical limitation to the broader implementation of this approach is the cost associated with transporting treated material from fixed treatment locations such as Culford Quarry. Investigation into the feasibility, operational constraints, and cost-benefit profile of a mobile metham sodium treatment system for use at remote gravel pits would represent a logical next step toward maximising the full environmental and logistical benefits of this research.

   (a) Scope the feasibility of a mobile metham sodium treatment system for treating gravel from known infested pits in remote natural areas, where transport costs, or limited access to nearby uninfested material may otherwise constrain asset-management activities such as track upgrades intended to protect high-value natural assets.

   (b) Develop a cost matrix for mobile treatment systems to allow land managers and project proponents to compare treatment costs against alternative transport costs and the environmental risks associated with deferred or unmanaged works.

   (c) Continue to investigate interstate adaptation, operational guidance, and broader adoption pathways involving multiple suppliers across Australia. Where required, develop jurisdiction-specific guidance that accounts for regional variation in soils, *Phytophthora* species, regulatory frameworks, and health, safety and environmental (HSE) requirements, including clear criteria defining when more conservative alternatives may be required.

3. Even during this short-term project, adjustments were implemented in response to emerging research relating to metham sodium behaviour and MITC generation. Alongside broader implementation of this treatment approach, there will be ongoing opportunities to contribute to the wider scientific understanding of metham sodium applications and address additional research questions identified through this project.

   (a) Undertake targeted research to more precisely define the minimum MITC exposure threshold (indicatively 700 mg·h/L) associated with reliable negative-recovery outcomes for *Phytophthora*. The potential application of this threshold within operational quality assurance frameworks and future optimisation studies should also be investigated.

(b) Continue to optimise metham sodium application rate, shroud design, and, perhaps most importantly, site layout. The 2025 trial showed good intra-stockpile MITC retention with a natural gravel crust on a 3 m high pile. Further improvements in exposure consistency and resilience to operational variability are therefore most likely to come from optimising the application system rather than additional stockpile sealing. Engineering-based approaches that minimise the distance between the mixing point and the delivery point, thereby delaying MITC and venting losses, are likely to improve effective fumigant delivery to the gravel matrix. Continued optimisation of spray box and shroud configuration, informed by existing loss-rate testing, may provide similar operational benefits.

(c) During the term of this project, new research was released indicating that both the dilution rate and pH of the water used for mixing are important factors influencing the rate at which the liquid metham sodium converts to MITC gas. Further research to optimise moisture conditions to support MITC generation and retention is therefore recommended. Proactive moisture management in future applications will help build resilience into the treatment design, reducing sensitivity to variations in initial moisture and weather conditions and supporting more consistent in-pile MITC exposure.

(d) Importantly, the identification of these research and optimisation opportunities does not diminish the operational significance of the current findings. Rather, these investigations provide a pathway to improve treatment consistency, strengthen quality assurance processes, and further reduce residual uncertainty. For example, the revised delivery rate developed during this project, adjusted using measured shroud-loss estimates to maintain the intended dosage rate applied to the gravel, could now be considered for incorporation into future revisions of MRWA operational guidance and associated policy documents.

7 Concluding statement

Overall, the results of this trial align strongly with findings from earlier proof-of-concept work conducted in 2019 and demonstrate that metham sodium treatment can substantially reduce detectable recovery of *P. cinnamomi* and *P. multivora* within infested laterite gravel under commercial-scale stockpile conditions. The trial provides a strong operational foundation for the use of metham sodium treatment as a risk-mitigation tool for reducing the likelihood of Phytophthora spread in civil engineering and infrastructure projects.

Residual positive recoveries observed in a small number of treated sample locations indicate that metham sodium fumigation should be considered within the context of an integrated Phytophthora dieback management framework, with treatment suitability informed by the sensitivity of adjacent environmental at-risk biodiversity assets and the acceptable level of residual risk. Ongoing monitoring and operational refinement will remain important for strengthening confidence in treatment performance and supporting appropriate long-term implementation.

8 References

Belhaj R, McComb J, Burgess TI, Hardy GSJ (2018) Pathogenicity of 21 newly described Phytophthora species against seven Western Australian native plant species. Plant Pathology 67:1140–1149

Burgess TI, López-Villamor A, Paap T, et al (2021) Towards a best practice methodology for the detection of Phytophthora species in soils. Plant Pathology 70:604–614

Davison EM, Kazemi S, McDonald S, et al (2019) Use of metham sodium to eliminate Phytophthora spp. from roading gravel. Australasian Plant Pathol 48:563–572. https://doi.org/10.1007/s13313-019-00660-0

Department of Biodiversity, Conservation and Attractions (2020) Phytophthora Dieback Management Manual. Department of Biodiversity, Conservation and Attractions, Perth

Department of Biodiversity, Conservation and Attractions (2023) Managing dieback risk associated with disturbance activities - Version 1.3. Government of Western Australia

Du B, Dechen T (2025) Laboratory evaluation of dilution factor and pH effects on the conversion of metham sodium to MITC. Harry Butler Institute, Murdoch University, Murdoch, Western Australia

Du B, Tshering D, Zhang P, et al (2026) Field analysis and monitoring of Methyl isothiocyanate (MITC) applied to the gravel stockpile trial 2025. Murdoch University (Harry Butler Institute), Murdoch, Western Australia

Hardy GSJ, Colquhoun IJ, Shearer BL, Tommerup IC (2001) The impact and control of Phytophthora cinnamomi in native and rehabilitated forest ecosystems in Western Australia. Forest Snow and Landscape Research 76:337–343

Main Roads Western Australia (2023) Specification 501: Pavements. Main Roads Western Australia

Nelson SD, Ajwa HA, Trout T, et al (2013) Water and methyl isothiocyanate distribution in soil after drip fumigation. Journal of Environmental Quality 42:1555–1564

Popp C, Grunewaldt-Stöcker G, Maiss E (2019) A soil-free method for assessing pathogenicity of fungal isolates from apple roots. Journal of Plant Diseases and Protection 126:329–341. https://doi.org/10.1007/s41348-019-00236-6

Tooley PW (1988) Use of uncontrolled freezing for liquid nitrogen storage of *Phytophthora* species. Plant Disease 72:680–682

Versalovic J (2011) Manual of clinical microbiology. American Society for Microbiology Press

Williams B, Eslick H (2019) Metham Sodium Fumigation of Main Roads Gravel - Method Revision B. ArborCarbon Report No. J19351-1:39

Appendix A: Gravel moisture assessment

Gravel moisture sampling plan and field procedure

Moisture content was assessed at two defined stages. Baseline moisture was measured from gravel sampled from the source stockpile before construction of the control and treated stockpiles. Post-construction/end-of-phase moisture was measured from gravel collected during dismantling of the control and treated stockpiles at the time of recovering the Burley cages, undertaken in accordance with HSE requirements and controls to minimise potential exposure to MITC during excavation and handling; corresponding results are reported in Control pile: ASM:MC25-02849 and Treated pile: ASM:MC25-02850.

Baseline moisture sampling

Sampling was completed on 28 August 2025 from the source gravel stockpile, prior to construction of the control and treated stockpiles, using a front-end loader to create costeans (trenches) to facilitate representative sampling in accordance with MRWA procedure WA100.1. Samples were collected by a NATA-accredited technician from Materials Consultants Pty Ltd.

Dismantling-phase moisture sampling

Sampling was completed while the control and treated stockpiles were being dismantled and pine plugs were being retrieved, by exposing the stockpile face using an excavator and collecting gravel from ~1 m in from the outer surface (locations designated as TS – Test Samples, Figure 13). This dismantling-phase sampling was scheduled in accordance with HSE requirements to minimise potential exposure to MITC during excavation and handling.

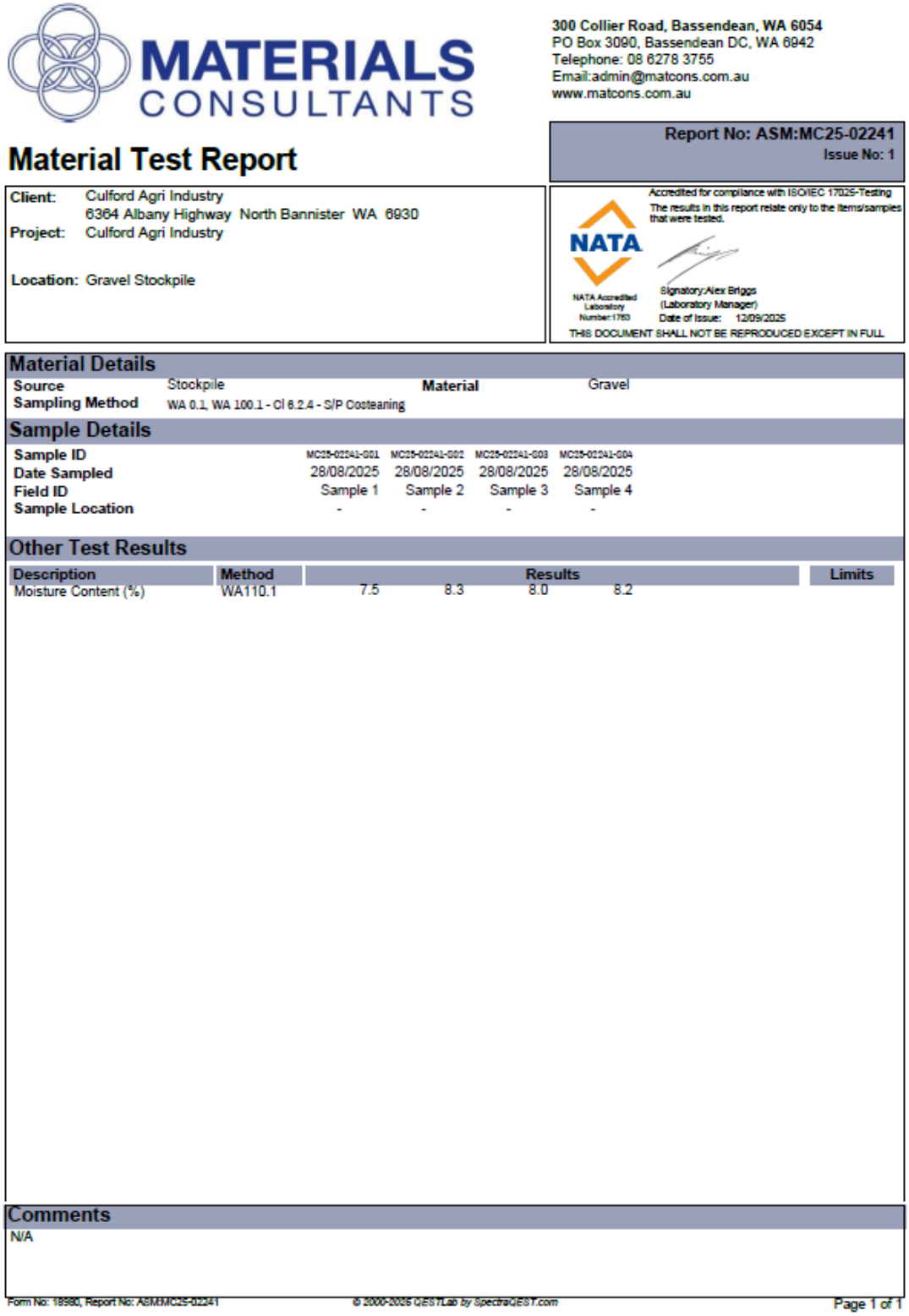
Sample handling and processing

Each moisture sample comprised approximately 1 kg (minimum) of gravel, placed into a new, clean plastic bag with the top twisted, folded over, and secured with an elastic band or cable tie to minimise moisture loss prior to testing. Samples were delivered to Materials Consultants Pty Ltd (or collected by arrangement) for the determination of moisture content in accordance with WA110.1. For dismantling-phase samples, the NATA report covered laboratory testing only (sampling was not undertaken by an accredited technician).

Figure 13. Moisture sampling locations for the control stockpile (a) and treated stockpile (b) twelve samples per stockpile were collected for moisture content analysis during deconstruction: one per corner (a and b), ensuring vertical distribution of sampling positions (c) At each sampling location, material was collected from approximately 1 m inward from the outer stockpile surface following excavation into the pile.

Appendix B: Gravel moisture data sheets

Full laboratory result tables are provided in ASM:MC25-02241, ASM:MC25-02849 and ASM:MC25-02850.



MATERIALS CONSULTANTS

300 Collier Road, Bassendean, WA 6054
PO Box 3090, Bassendean DC, WA 6942
Telephone: 08 6278 3755
Email:admin@matcons.com.au
www.matcons.com.au

Material Test Report

Report No: ASM:MC25-02241
Issue No: 1

Client: Culford Agri Industry
6364 Albany Highway North Bannister WA 6930
Project: Culford Agri Industry

Location: Gravel Stockpile

Accredited for compliance with ISO/IEC 17025-Testing
The results in this report relate only to the items/samples that were tested.

NATA Accredited Laboratory Number:1763

Signatory:Alex Briggs
(Laboratory Manager)
Date of Issue: 12/09/2025

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Material Details

Source	Stockpile	**Material**	Gravel
Sampling Method	WA 0.1, WA 100.1 - Cl 6.2.4 - S/P Costeaning		

Sample Details

Sample ID	MC25-02241-S01	MC25-02241-S02	MC25-02241-S03	MC25-02241-S04
Date Sampled	28/08/2025	28/08/2025	28/08/2025	28/08/2025
Field ID	Sample 1	Sample 2	Sample 3	Sample 4
Sample Location	-	-	-	-

Other Test Results

Description	Method	Results				Limits
Moisture Content (%)	WA110.1	7.5	8.3	8.0	8.2	

Comments

N/A

Form No: 18980, Report No: ASMMC25-02241 © 2000-2025 QES7Lab by SpectraQES7.com Page 1 of 1

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300 Collier Road, Bassendean, WA 6054
PO Box 3090, Bassendean DC, WA 6942
Telephone: 08 6278 3755
Email:admin@matcons.com.au
www.matcons.com.au

Material Test Report

Report No: ASM:MC25-02849
Issue No: 1

Client: Dieback Working Group
PO Box 198 Fremantle WA 6959
Project: Culford Agri Industry, North Bannister, WA

Location: Laterite gravel from Control stockpile

Accredited for compliance with ISO/IEC 17025-Testing
The results in this report relate only to the items/samples that were tested.

NATA Accredited Laboratory Number:1783

Signatory:Alex Griggs
(Laboratory Manager)
Date of Issue: 6/11/2025

THIS DOCUMENT SHALL NOT BE REPRODUCED EXCEPT IN FULL

Material Details

Source Stockpile **Sampling Method** Tested as received

Sample Details

Sample ID	MC25-02849-S01	MC25-02849-S02	MC25-02849-S03	MC25-02849-S04
Date Sampled	3/11/2025	3/11/2025	3/11/2025	3/11/2025
Client ID	TS1-A (Control)	TS1-B	TS1-C	TS2-A (Control)
Sample Location	-	-	-	-
Soil Description	Laterite Gravel	Laterite Gravel	Laterite Gravel	Laterite Gravel

Other Test Results

Description	Method	Results				Limits
Moisture Content (%)	WA110.1	9.4	8.6	9.5	9.6	

Comments

N/A

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300 Collier Road, Bassendean, WA 6054
PO Box 3090, Bassendean DC, WA 6942
Telephone: 08 6278 3755
Email:admin@matcons.com.au
www.matcons.com.au

Material Test Report

Report No: ASM:MC25-02849
Issue No: 1

Client: Dieback Working Group
PO Box 198 Fremantle WA 6959
Project: Culford Agri Industry, North Bannister, WA

Location: Laterite gravel from Control stockpile

Accredited for compliance with ISO/IEC 17025-Testing
The results in this report relate only to the items/samples that were tested.

NATA Accredited Laboratory Number:1783

Signatory:Alex Briggs
(Laboratory Manager)
Date of Issue: 6/11/2025

THIS DOCUMENT SHALL NOT BE REPRODUCED EXCEPT IN FULL

Material Details

Source	Stockpile	**Sampling Method**	Tested as received

Sample Details

Sample ID	MC25-02849-S05	MC25-02849-S06	MC25-02849-S07	MC25-02849-S08
Date Sampled	3/11/2025	3/11/2025	3/11/2025	3/11/2025
Client ID	TS2-B	TS2-C	TS3-A (Control)	TS3-B
Sample Location	-	-	-	-
Soil Description	Laterite Gravel	Laterite Gravel	Laterite Gravel	Laterite Gravel

Other Test Results

Description	Method	Results				Limits
Moisture Content (%)	WA110.1	9.7	9.6	8.8	8.7	

Comments

N/A

MATERIALS CONSULTANTS

300 Collier Road, Bassendean, WA 6054
PO Box 3090, Bassendean DC, WA 6942
Telephone: 08 6278 3755
Email:admin@matcons.com.au
www.matcons.com.au

Material Test Report

Report No: ASM:MC25-02849
Issue No: 1

Client: Dieback Working Group
PO Box 198 Fremantle WA 6959
Project: Culford Agri Industry, North Bannister, WA

Location: Laterite gravel from Control stockpile

Accredited for compliance with ISO/IEC 17025-Testing
The results in this report relate only to the items/samples that were tested.

NATA Accredited Laboratory Number:1783

Signatory:Alex Briggs
(Laboratory Manager)
Date of Issue: 6/11/2025

Material Details

Source Stockpile **Sampling Method** Tested as received

Sample Details

Sample ID	MC25-02849-S09	MC25-02849-S10	MC25-02849-S11	MC25-02849-S12
Date Sampled	3/11/2025	3/11/2025	3/11/2025	3/11/2025
Client ID	TS3-C	TS4-A (Control)	TS4-B	TS4-C
Sample Location	-	-	-	-
Soil Description	Laterite Gravel	Laterite Gravel	Laterite Gravel	Laterite Gravel

Other Test Results

Description	Method	Results				Limits
Moisture Content (%)	WA110.1	8.6	9.5	9.3	8.7	

Comments

N/A

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300 Collier Road, Bassendean, WA 6054
PO Box 3090, Bassendean DC, WA 6942
Telephone: 08 6278 3755
Email:admin@matcons.com.au
www.matcons.com.au

Material Test Report

Report No: ASM:MC25-02850
Issue No: 1

Client: Dieback Working Group
PO Box 198 Fremantle WA 6959
Project: Culford Agri Industry, North Bannister, WA

Location: Laterite gravel from Trial stockpile

Accredited for compliance with ISO/IEC 17025-Testing
The results in this report relate only to the items/samples that were tested.

NATA Accredited Laboratory Number:1783

Signatory:Alex Briggs
(Laboratory Manager)
Date of Issue: 6/11/2025

Material Details

Source Stockpile **Sampling Method** Tested as received

Sample Details

Sample ID	MC25-02850-S01	MC25-02850-S02	MC25-02850-S03	MC25-02850-S04
Date Sampled	3/11/2025	3/11/2025	3/11/2025	3/11/2025
Client ID	TS5-A	TS5-B	TS5-C	TS6-A
Sample Location	-	-	-	-
Soil Description	Laterite Gravel	Laterite Gravel	Laterite Gravel	Laterite Gravel

Other Test Results

Description	Method	Results				Limits
Moisture Content (%)	WA110.1	9.6	9.5	9.1	9.8	

Comments

N/A

MATERIALS CONSULTANTS
300 Collier Road, Bassendean, WA 6054
PO Box 3090, Bassendean DC, WA 6942
Telephone: 08 6278 3755
Email:admin@matcons.com.au
www.matcons.com.au

Material Test Report

Report No: ASM:MC25-02850
Issue No: 1

Client: Dieback Working Group
PO Box 198 Fremantle WA 6959
Project: Culford Agri Industry, North Bannister, WA

Location: Laterite gravel from Trial stockpile

Accredited for compliance with ISO/IEC 17025-Testing
The results in this report relate only to the items/samples that were tested.

NATA Accredited Laboratory Number:1783

Signatory:Alex Briggs
(Laboratory Manager)
Date of Issue: 6/11/2025

Material Details

Source	Stockpile	**Sampling Method**	Tested as received

Sample Details

Sample ID	MC25-02850-S05	MC25-02850-S06	MC25-02850-S07	MC25-02850-S08
Date Sampled	3/11/2025	3/11/2025	3/11/2025	3/11/2025
Client ID	TS6-B	TS6-C	TS7-A	TS7-B
Sample Location	-	-	-	-
Soil Description	Laterite Gravel	Laterite Gravel	Laterite Gravel	Laterite Gravel

Other Test Results

Description	Method	Results				Limits
Moisture Content (%)	WA110.1	10.1	9.3	10.0	10.8	

Comments

N/A

MATERIALS CONSULTANTS
300 Collier Road, Bassendean, WA 6054
PO Box 3090, Bassendean DC, WA 6942
Telephone: 08 6278 3755
Email:admin@matcons.com.au
www.matcons.com.au

Material Test Report

Report No: ASM:MC25-02850
Issue No: 1

Client: Dieback Working Group
PO Box 198 Fremantle WA 6959
Project: Culford Agri Industry, North Bannister, WA

Location: Laterite gravel from Trial stockpile

Accredited for compliance with ISO/IEC 17025-Testing
The results in this report relate only to the items/samples that were tested.

NATA Accredited Laboratory Number:1783

Signatory:Alex Briggs
(Laboratory Manager)
Date of Issue: 6/11/2025

Material Details

Source	Stockpile	**Sampling Method**	Tested as received

Sample Details

Sample ID	MC25-02850-S09	MC25-02850-S10	MC25-02850-S11	MC25-02850-S12
Date Sampled	3/11/2025	3/11/2025	3/11/2025	3/11/2025
Client ID	TS7-C	TS8-A	TS8-B	TS8-C
Sample Location	-	-	-	-
Soil Description	Laterite Gravel	Laterite Gravel	Laterite Gravel	Laterite Gravel

Other Test Results

Description	Method	Results				Limits
Moisture Content (%)	WA110.1	10.3	8.6	10.2	8.1	

Comments

N/A

Appendix C: Intergranular MITC summary table

This appendix presents the intergranular (in-stockpile) MITC monitoring results reported by Du et al. (2026) for the treated stockpile and selected control points (Table 10). Results are summarised by sampling point ID and include the number of sampling events (n), descriptive statistics for measured MITC concentrations (mean, SD, minimum and maximum; ppm), and cumulative exposure (C×T; mg·h/L). Values reported as <LoD were treated as zero for the descriptive summaries. These data are provided to document within-pile variability in MITC exposure and to support exploratory comparison with pine plug sterilisation outcomes where spatial alignment between monitoring points and plug locations is defensible.

Table 10. Probe-level summary of intergranular MITC monitoring results by stockpile and sampling point (Du et al. 2026). MITC concentrations (ppm) from fixed gas-sampling points are summarised across sampling events as mean, SD, minimum and maximum. Values reported as below the level of detection were treated as zero for these descriptive statistics. Cumulative exposure (C×T; mg·h/L) is reported for each sampling point.

Stockpile	MITC point	n (events)	Mean (ppm)	SD	Min	Max	C×T (mg·h/L)
Control	C-1A1	20	0	0	0	0	0
Control	C-16D1	20	0	0	0	0	0
Control	C-19C1	20	0	0	0	0	0
Treated	T-3A1	20	1.81	6.23	0	28.87	2809.4
Treated	T-3A2	20	0.39	1.00	0	4.64	769.7
Treated	T-3B1	20	1.32	3.05	0	13.51	2175.7
Treated	T-3B2	20	5.46	21.57	0	99.43	8122.8
Treated	T-3C1	20	0.34	0.36	0	1.27	760.7
Treated	T-3C3	20	0.22	0.29	0	1.08	512.7
Treated	T-6A3	20	0.34	0.60	0	2.54	769.1
Treated	T-6A4	20	0.26	0.27	0	0.93	611.4
Treated	T-6B3	20	0.38	0.49	0	1.68	955.8
Treated	T-6C1	20	0.58	0.70	0	2.63	1553.2
Treated	T-6C4	20	0.23	0.35	0	1.22	642
Treated	T-6D1	20	0.47	0.54	0	1.87	1118
Treated	T-9A1	20	0.63	0.68	0	2.64	1519.7
Treated	T-9A2	20	0.80	0.72	0	2.62	1988.3
Treated	T-9B1	20	1.07	1.07	0	3.65	2604.9
Treated	T-9B2	20	1.58	1.56	0.03	5.37	3754
Treated	T-9C2	20	0.77	0.83	0	2.36	2070.1
Treated	T-9C3	20	0.63	0.74	0	2.76	1956
Treated	T-12A3	16	1.04	1.07	0	3.86	2341.1
Treated	T-12A4	16	1.13	1.11	0	3.36	2388.6
Treated	T-12B3	16	1.80	1.67	0.02	5.09	4161.9
Treated	T-12C1	16	1.39	1.43	0	4.00	2837.6
Treated	T-12C4	16	2.03	2.01	0	6.30	4184.4
Treated	T-12D1	16	0.83	0.81	0	2.60	1825.5
Treated	T-15A1	16	1.56	1.52	0	4.70	3113.4
Treated	T-15A2	16	1.10	0.91	0	2.60	2531.5
Treated	T-15B1	16	1.59	1.24	0	3.77	3878.9
Treated	T-15B2	16	1.27	1.05	0	3.39	2818
Treated	T-15C2	16	1.13	0.89	0	2.71	2937.6

Stockpile	MITC point	n (events)	Mean (ppm)	SD	Min	Max	C×T (mg·h/L)
Treated	T-15C3	16	1.46	1.24	0	3.67	3498.2
Treated	T-18A3	16	0.44	0.51	0	1.68	1189.2
Treated	T-18A4	16	0.46	0.43	0	1.34	1334.8
Treated	T-18B3	16	0.72	0.69	0	2.23	1799.1
Treated	T-18C1	16	0.36	0.26	0	0.91	1005.9
Treated	T-18C4	16	0.92	0.66	0.06	2.22	2708.8
Treated	T-18D1	16	0.26	0.24	0	0.73	661.7