Hort Innovation

Final Report

Improved management of charcoal rot of strawberry

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Delivery partner:

Department of Agriculture and Fisheries

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BS15005

Project:

Improved management of charcoal rot of strawberry BS15005

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Content

Summary

A three-year research project undertaken by the Department of Agriculture and Fisheries (DAF) and the Victorian Strawberry Industry Certification Authority (VSICA), and co-funded through Hort Innovation, has led to new practices enabling strawberry growers to improve the control of charcoal rot.

Charcoal rot is a major disease of strawberry capable of causing devastating plant deaths and has a substantial financial impact through lost income. Charcoal rot is a soil borne disease caused by the fungus *Macrophomina phaseolina* and has been found in strawberries in all states of Australia, although not all strawberry production districts.

The new practices and key findings from the research include:

The use of totally impermeable film (TIF) with fumigants reduced plant deaths due to charcoal rot by 91% (i.e. average plant deaths after shank injected Tri-Form® 80 was 1.7% for TIF and 20% for LDPE). The improved control is achieved by the TIF plastic retaining the chemical fumigants in the soil at higher concentrations (up to 32%) for a longer period, compared with the industry standard low-density polyethylene (LDPE) plastic mulch. TIF is cost effective. Income from the additional fruit harvested exceeds the additional cost of TIF, resulting in an increase in gross income by up to $1.49/plant for the season. TIF is a superior plastic mulch for use with chemical soil fumigants and has been quickly adopted by strawberry growers in Victoria (>80%), South Australia (>70%) and Western Australia (>35%).

Research from the project has shown that the pathogen causing charcoal rot will survive in buried strawberry crowns from one season to the next. For example, *M. phaseolina* survived in crowns retained in the paddock for at least six months in a sub-tropical (Sunshine Coast, Queensland) and 13 months in a temperate environment (Granite Belt, Queensland). Standard industry practice has been to return infected strawberry crowns to the soil at the end of the fruiting season.

M. phaseolina surviving in infected crowns from the previous crop were shown to infect new strawberry plants within weeks of planting, with up to 50% of plants dead after eight weeks. Consequently, infected crowns from the previous crop are a major source of inoculum leading to outbreaks of charcoal rot in the following season. On learning of this at our communication events, some Australian growers have started to remove previous crop plant debris (Australian Berry Journal, Edition 3, 2020).

The results of field research in the project contributed to the registration (2018) of EDN Fumigas™ for use in strawberry. Chemical fumigants not yet registered in Australia were also evaluated to identify possible options for growers into the future.

The chemical fumigants Tri-Form® 80 (80% chloropicrin, 20% 1, 3-dichloropropene) and EDN Fumigas™ (ethanedinitrile) reduced plant deaths due to charcoal rot by 83% and 95% respectively, compared with untreated soil (i.e. average plant deaths due to charcoal rot was 7.8% for blocks treated with Tri-Form® 80, 2.2% for blocks treated with EDN and 47.8% for untreated). This result has led to significant numbers of growers in Queensland, South Australia and Western Australia adopting these fumigant products. As of 2020, 98% of strawberry growers in Victoria have adopted either Tri-Form® 80 or EDN Fumigas™ in place of chloropicrin (previous standard), compared to <5% in 2017.

Fumigant application techniques were shown to improve charcoal rot control. Directly injecting the chemical fumigant into the soil by shank fumigation reduced plant deaths due to charcoal rot by up to 37% compared with drip applied fumigation (i.e. average plant deaths following fumigation with EDN under TIF was 5% for shank injection compared to 8% for drip applied). The adoption of shank application of fumigants by strawberry growers in Western Australia has increased from <10% in 2017 to >33% of the production area in 2020, as a result. Field experiments showed that broad-acre fumigation under LDPE of the entire paddock reduced deaths due to charcoal rot by 66% compared to strip fumigation of the raised beds under LDPE (i.e. average plant deaths due to charcoal rot was 7% for blocks treated with broadacre fumigation and 20% for blocks treated with strip fumigation).

Strawberry growers across Australia were regularly informed of the benefits of farm biosecurity practices through many communications events. Our farm surveys in 2020 showed that 40% of Victorian strawberry growers had adopted at least one new farm biosecurity practice since 2017. The adoption of these improved biosecurity practices is predicted to reduce the spread of charcoal rot across Australian strawberry farms.

Crop termination, the process of destroying an old strawberry crop with metham sodium followed by pre-plant fumigation, increased fruit yields by 30% and revenue by $1.29/plant in the following season, compared with pre-

plant fumigation alone. Based on these results, several strawberry growers are planning to trial crop termination on their properties during 2020-2021.

An extensive programme of information delivery activities was undertaken throughout the 3-year project to ensure that growers and industry participants received the latest news about research progress and practices for controlling charcoal rot. Project team members delivered multiple presentations at 41 events in five states, along with 14 articles in industry publications. These communication outputs have led to the increased adoption of improved farm practices by Australian strawberry growers and resulted in a 5% reduction in charcoal rot on Victorian strawberry farms from 2017 to 2020. This translates to an estimated $5M additional revenue in 2020 from improved management of charcoal rot in the Victorian industry alone.

The research has delivered several practices enabling an immediate improvement in charcoal rot control by strawberry growers. In addition, the project work has identified aspects in managing charcoal rot that require future research, including biofumigants and other non-chemical controls, and the evaluation of diagnostic tests to support growers in the selection and use of the most cost-effective treatments.

Keywords

Strawberry, charcoal rot, *Macrophomina phaseolina*, fumigation, soil-borne disease

Introduction

Strawberry (*Fragaria x ananassa* Duchesne) is an important crop in Australia. In the 2015 season, Australia produced 77,000 tonnes of strawberry fruit valued at more than $420M (Australian Horticultural Statistics Handbook 2014/15, Horticulture Innovation Australia). Increasing occurrences of charcoal rot after 2005, when methyl bromide was withdrawn from use as a soil fumigant in Australia, saw the disease become a serious problem for strawberry growers. Charcoal rot can kill large numbers of strawberry plants and the lack of effective controls saw the disease become a significant financial threat for industry.

Charcoal rot is caused by the soil-borne fungus *Macrophomina phaseolina* (Tassi) Goid. It has recently been reported in strawberry fruit crops in Queensland, Victoria, South Australia, Tasmania and Western Australia (Hutton et al., 2013; Golzar et al., 2007), where it has caused plant losses of up to 50%. A comprehensive field survey conducted in 2017 showed that *M. phaseolina* was detected in soil at 80% of all strawberry properties in Victoria. Similarly, charcoal rot has been reported in strawberry crops in Spain, the United States, Israel, Turkey, and Argentina (Aviles et al., 2007; Baino et al., 2011; Koike, 2008; Mertley et al., 2005; Yildiz et al., 2010; Zveibel and Freeman, 2005).

Soil fumigation with a mixture of methyl bromide (MB) and chloropicrin (Pic) controls charcoal rot in strawberries (Hutton et al, 2013). Research in Australia showed that only a 50:50 mixture of MB:Pic could eradicate *M. phaseolina* in infected crowns in the soil (Hutton et al., 2013) and that fumigants widely used in the strawberry industry (chloropicrin and 1,3 dichloropropene (1,3-D)) were not effective. It is likely that the continued use of MB:Pic in the Victorian sector of the strawberry nursery industry under a critical-use exemption has prevented outbreaks of *M. phaseolina* in strawberry runner crops in Australia. The strawberry fruit industry had an urgent need for soil fumigants to replace MB:Pic. Field research was undertaken to evaluate registered and new fumigants for effectiveness in reducing inoculum of *M. phaseolina* in soil and in crop residues in soil using different plastic mulch (e.g. impermeable barrier films), combinations of products (e.g. chloropicrin followed by metham sodium), formulations (e.g. 1,3-dichloropropene/chloropicrin containing higher concentrations of chloropicrin), and application techniques (e.g. shank injection). Research in this project tested the hypothesis that improved application techniques for current fumigants or new fumigants can manage *M. phaseolina* and charcoal rot in strawberry more effectively than existing industry practices (i.e. fumigants with unproven effectiveness against *M. phaseolina* and/or low-density polyethylene (LDPE).

Infected strawberry crowns, alternative hosts and weeds in cultivated strawberry land, are potential sources of inoculum adding to the risk of charcoal rot outbreaks. The fungus infects a wide range of hosts, including many cultivated crops (Mass, 1998) and weeds (Fuhlbohm et al., 2011). Research in California indicated that *M. phaseolina* isolates from strawberry may have a host preference for strawberry, and isolates tested from alternative host crops (watermelon, thyme and apple) did not cause disease in strawberry (Koike et al., 2016). However, studies in Israel found *M. phaseolina* isolates from strawberry and several other crops (almond, aralia, protea, melon and watermelon) affected strawberry plants similarly (Zveibil et al., 2012). The authors highlighted the non-specificity of *M. phaseolina* isolates and potential of other crops to contribute to soil inoculum levels of *M. phaseolina*. A recent study in Queensland also supports the ability of non-strawberry isolates of *M. phaseolina* to infect strawberry (Gomez, 2020), suggesting non-strawberry hosts of *M. phaseolina* need to be included in a charcoal rot management strategy on strawberry farms.

At the end of the strawberry harvest season, it is common industry practice for plants to be incorporated back into the soil. Crop residues infected with charcoal rot can perpetuate the pathogen in the field and may increase inoculum levels each year (Dhingra and Sinclair, 1978). *M. phaseolina* survives in soil and crop residues as microsclerotia (Bolda and Koike, 2010). Once the host tissue degrades, microsclerotia are released in the soil (Cook et al, 1973). Several authors have reported *M. phaseolina* microsclerotia can survive in soil between 2-15 years (cited in Sarr et al., 2014). In bean, the fungus was recovered from crop debris buried in soil after 21 months (Songa and Hillocks, 1998). It is not known how long *M. phaseolina* can survive in strawberry crown debris. Tillage practices and soil environment conditions (temperature, moisture) may also affect the survival of the fungus. Farm practices that promote the destruction of plant residue may directly or indirectly lower the longevity of *M. phaseolina* (Baird et al., 2003). Also, Cook et al. (1973) found crop residue not incorporated into the soil will delay the decaying process, and provide the fungus maximum protection and longevity in plant residue. The research results suggest that managing infected crop debris and alternative host crops can reduce *M. phaseolina* populations in soil and minimise the severity of charcoal rot in strawberry.

Farm biosecurity practices can reduce the spread of soil-borne pathogens within and between properties. For

example, washing down farm equipment and vehicles to remove soil before entering the farm or moving between paddocks is integral to containing the spread of soil-borne pathogens. At the start of the project, strawberry growers were complacent about farm biosecurity and few employed farm hygiene practices in daily operations. Adoption of farm biosecurity practices across the strawberry industry has the potential to add to the control of charcoal rot.

The objective of this research project was to develop improved management options for charcoal rot in strawberry fruit crops, including farm biosecurity, chemical and cultural options. The project aligns with Strategic Outcome 3.4 of the Strawberry Strategic Investment Plan 2017-2021.

Methodology

This research project is relevant to growers, consultants and service providers within the strawberry industry across Australia, and particularly those individuals currently affected by charcoal rot. The project draws upon overseas research and the outputs will be of interest to scientists working on the topic by adding to our knowledge of charcoal rot.

Research in this project used a series of methods to rapidly screen combinations of 20 fumigant products, 10 application techniques and 8 cultural practices for their effectiveness against charcoal rot (Appendix I). Soil columns were used to initially screen chemical fumigants and application techniques. The effectiveness of each fumigation treatment was verified in field experiments in commercial strawberry production systems. This approach culminated in a field experiment to evaluate an integrated strategy of the best chemical and cultural practices for control of charcoal rot in the field. A combination of communication methods were used to engage industry and deliver information to growers and other users (i.e. chemical companies, fumigant contractors, agronomists, industry development officers, plastics manufacturers and distributors). The project team implemented the following methodological approaches to maximise their time and resources to improve the management of charcoal rot by strawberry growers:

Soil column experiments

Soil column experiments are an established method for rapidly screening fumigant products and application techniques under different soil and environmental conditions (Zheng *et al*. 2006, Ashworth and Yates 2007). The method is particularly useful for understanding how fumigants move through soil, and how this relates to pathogen control. We conducted six soil column experiments to assess the efficacy of different rates, formulations, sealing methods (totally impermeable film (TIF) and low-density polyethylene film (LDPE)) and fumigant products (including Tri-From® 80 and similar products, dimethyl disulphide, methyl iodide, metham sodium and Pic Plus® Fumigant) against *M. phaseolina* in soil and crop residues (Appendix I). The relatively short time (one month) to complete a soil column experiment allowed us to quickly select the most promising treatments for inclusion in field trials. It also generated new information that was delivered to growers early in the project.

Inoculum experiments

Prior to this project, it was not known how long *M. phaseolina* survived in buried strawberry crop residue. Such knowledge is fundamental for managing charcoal rot, as standard industry practice is to incorporate strawberry plants back in the soil after the season. Two field experiments were conducted to determine the longevity of *M. phaseolina* in buried strawberry crowns. One of these experiments was conducted in a temperate environment (Granite Belt, QLD) while the other was conducted in a sub-tropical environment (Sunshine coast, QLD). The duration of each experiment approximated the interval between strawberry crops in the two different growing environments.

Transmission of *M. phaseolina* inoculum surviving in strawberry crop debris to new plants was studied in a pot experiment under controlled conditions in a glasshouse. The project also investigated the impact of the quantity and type of crown material (whole or cut) on the timing of infection and rate of disease development.

Non-strawberry (alternative) hosts of *M. phaseolina* are a potential additional source of inoculum in strawberry paddocks. Dozens of weed and crop species were collected from strawberry farms in Queensland and Victoria to identify those species infected with *M. phaseolina*. This approach was not comprehensive in identifying all alternative hosts of *M. phaseolina,* as only those species present at the time of sampling were screened for *M. phaseolina* infection.

Field experiments

Field experiments are an established method for comparing the efficacy of new disease control practices with standard industry practices under commercial production conditions. Eight field experiments were used to assess the efficacy of fumigants (Tri-From® 80, Metham sodium, EDN™ Fumigas, Agrocelhone® FE, Basamid® Granular, Methyl bromide recaptured from quarantine applications on carbon, DOMINUS® and/or Pic Plus® Fumigant), plastic mulches (TIF or LDPE, black or silver TIF), fumigant application techniques (strip or broadacre fumigation, shank injection or drip-line fumigation, and crop termination), planting material (crown size, plugs or bare-rooted transplants) and/or the removal of crop residue in the control of *M. phaseolina* and charcoal rot. Fumigant concentrations were recorded during several of these experiments to compare treatments (Appendix I). A final field experiment evaluated the efficacy of an integrated management programme combining the most efficacious fumigant treatments and cultural practices identified in the initial project research. Fruit yield data collected for

the entire season at the field experiments enabled economic analyses to compare the cost effectiveness of new treatments with standard industry practices. Increased returns identified by economic analyses were important in driving the adoption of new management practices across industry.

Farm biosecurity

Farm hygiene practices for soil-borne diseases in other crops were adjusted to suit the operational procedures of Australian strawberry growers whilst retaining their efficacy. Detailed information on individual farm biosecurity practice was delivered to strawberry growers at the many communication events throughout the project.

Information delivery

Multiple communication methods were employed to deliver information from the research in this project to growers and other users. These included: industry magazine articles, conference papers, oral (PowerPoint) presentations, field days and workshops with participatory activities such as soil column demonstrations. The pathogen became real (tangible) for growers when they viewed *M. phaseolina* under the microscope and a soil column demonstration of the retention of fumigant under TIF reinforced the key message in articles and oral presentations. Farm visits enabled project team members to apply the key messages to the features of the individual growers' production system. The project team participated in communication events facilitated by fumigators, plastics manufactures, and agronomists as an additional conduit for delivering information and promoting the adoption of improved practices to growers. These individuals and their respective companies continue to provide information from the national project to growers across the country. Response cards were used at several communication events to receive and record grower feedback during presentations, including their preferred method for obtaining scientific information. One of the messages from this method was that >90% of growers prefer receiving information in small groups compared with any other delivery method.

Outputs

During this project, project team members have delivered and presented on project activities and results at industry meetings and events held locally, nationally, and internationally.

- More than 30 presentations and demonstrations at strawberry industry events convened by industry associations, commercial suppliers or the project research partners (DAF and VSICA) for more than 550 grower contacts, researchers, and industry affiliates nationally and internationally.
- Presented at 8 national and international conferences and interacted with over 1500 national and international delegates (such as growers, fumigators, government representatives, researchers, and academics).
- Wrote 13 articles for the national industry magazine (Simply Red and Australian Berry Journal), distributed to more than 650 growers and industry associates per edition. Five Hortlink updates were also published on the Horticulture Innovation Australia website during the project.
- Authored 12 peer-reviewed manuscripts [Refer to Referred Scientific Publication section]

Grower and industry affiliates meetings, presentations, and workshops

2017

- Victorian Strawberry Growers Association annual general meeting, Silvan, Victoria (October)
- Meeting with Richdale Plastics (plastic manufacturers), strawberry growers and fumigators, Mentone, Victoria (October)
- Queensland Strawberry Growers' Association meeting, Glasshouse Mountains, Queensland (October)
- Victorian Strawberry Industry Certification Authority (VSICA) meeting with Plant Biosecurity Co-operative Research Centre (PBCRC) board and staff, Toolangi, Victoria (December)
- VSICA annual general meeting, Toolangi, Victoria, (December)

2018

- Field demonstration of totally impermeable film (TIF) plastic, Coldstream, Victoria (February)
- Meeting and field trials visits with TriCal representatives and fumigators, Silvan and Wandin, Victoria (February)
- Project staff meeting and field trial visits, Wandin, Victoria (February)
- Methyl Bromide Technical Options Committee (MBTOC) field trial visits, Coldstream, Victoria (March)
- Victorian roadshow communication event, Gruyere, Victoria (May)
- Victorian roadshow communication event, Silvan, Victoria (May)
- Victorian roadshow communication event, Coldstream, Victoria (May)
- Victorian roadshow communication event, Wandin, Victoria (May)
- Victorian roadshow communication event, Wandin North, Victoria (May)
- Victorian strawberry forum, Wandin North, Victoria (May)
- Victorian roadshow communication event, Cobram, Victoria (June)
- Victorian roadshow communication event, Bachus Marsh, Victoria (June)
- Victorian roadshow communication event, Mornington, Victoria (June)
- Queensland strawberry industry workshop and seminar, Stanthorpe, Queensland (October)
- VSICA Annual General Meeting, Toolangi, Victoria (November)
- South Australia Strawberry Farms Survey, Adelaide Hills, South Australia (December)

2019

- Western Australia National Masterclass and Strawberry Farms Survey, Wanneroo, Western Australia (February)
- Temperate Strawberry Forum, Wandin, Victoria (June)
- R&R (Australian fumigator company) Workshop, Yarra Glen, Victoria (July)

- Meeting and trial site visits with New Zealand industry representatives, Coldstream, Victoria (July)
- Queensland strawberry industry workshop and seminar, Stanthorpe, Queensland (September)
- Meeting with TriCal representatives, Gilroy, California (November)
- Meeting with TriCal Diagnostics representatives, Hollister, California (November)
- Meeting with strawberry industry representatives (runner certification and breeding program), University of California Davis, Davis, California (November)
- Victorian strawberry growers field day, Wandin, Victoria (December)

2020

- Queensland Strawberry Grower's meeting, Caboolture, Queensland (July)
- Department of Agriculture and Fisheries Sub-Tropical Fruits and Genetic Improvement team meeting (virtual meeting), Brisbane, Queensland (August)

Conferences

- Plant Biosecurity Cooperative Research Centre (PBCRC) Conference presentation, Melbourne, Victoria, September 2017
- Science Protecting Plant Health, Australasian Plant Pathology Conference, Brisbane, Queensland, September 2017
- Methyl Bromide Alternatives Outreach Conference, San Diego, California, USA, November 2017
- BerryQuest conference, Launceston, Tasmania, February 2018
- PBCRC National Science Exchange, Melbourne, Victoria, May 2018
- Australasian Soilborne Disease Symposium, Adelaide, South Australia, September 2018
- Methyl Bromide Alternatives Outreach Conference, San Diego, California, USA, November 2019
- Australasian Plant Pathology Society conference, Melbourne, Victoria, November 2019
- Methyl Bromide Alternatives Outreach Conference, San Diego, California, USA, (virtual conference) November 2020

Articles

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McFarlane, D., Mattner, S., Greenhalgh F. C., Gomez, A. & Oag, D. 2018. Totally impermeable films increase fumigant concentrations in soil. *Simply Red*, 50, 3-5.

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Oag, D. 2019. Charcoal rot – A disease of strawberry and blueberry. *Australian Berry Journal*, 1, 43-45.

Oag, D., Gomez, A., McFarlane, D. & Mattner, S. 2019. Retaining infected crop debris leads to charcoal rot. *Simply Red*, 54, 7.

Oag, D., Gomez, A., McFarlane, D. & Mattner, S. 2020. New practices for Improved control of charcoal rot. *Australian Berry Journal*, 3, 57-59.

Project team members undertook and participated in numerous activities engaging growers with information generated from the project (Figures 1-8)

Figure 1. Dylan M^{c}Farlane demonstrating the collection and measuring of fumigant from soil columns, Applethorpe, Queensland, Sept 2018

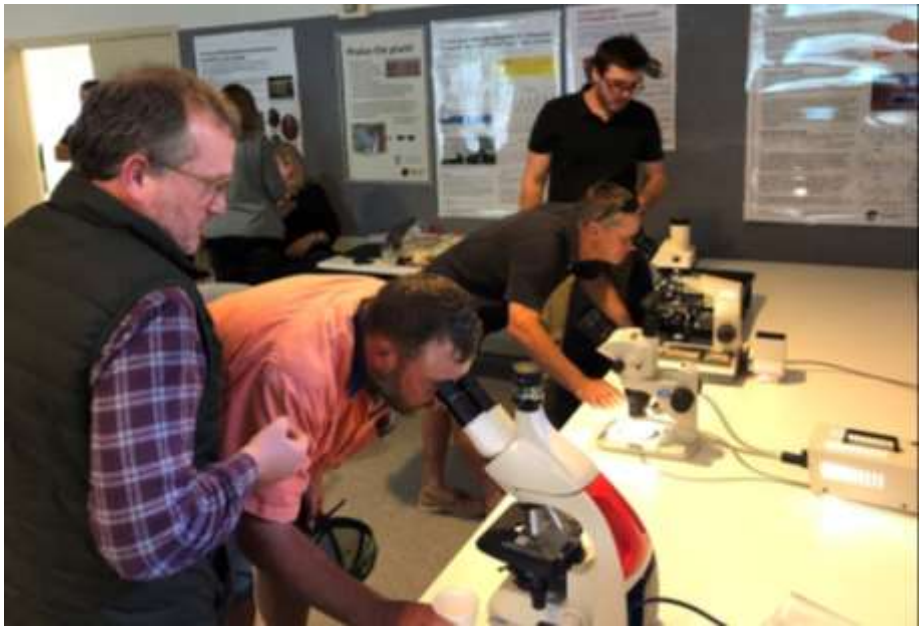

Figure 2. Scott Mattner (L) and Dylan M^{c}Farlane showing growers the *Macrophomina* pathogen under the microscopes, Applethorpe, Queensland, Sept 2018

Figure 3. Project team members with Mr Bruno de Ingeniis (Yarra Valley Farms), Yarra Valley, Victoria, Feb 2018

Figure 4. David Oag introducing the project at the BerryQuest conference, Launceston, Tasmania, Feb 2018

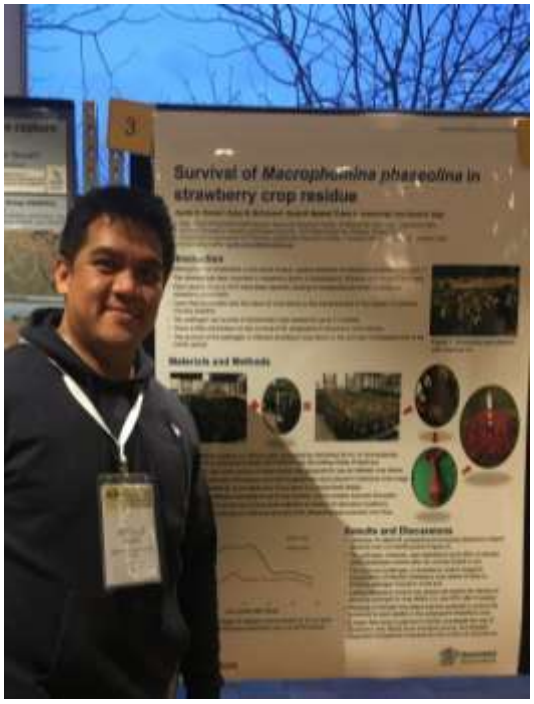

Figure 5. Apollo Gomez at the Australasian Soilborne Disease Symposium, Adelaide, South Australia, Sept 2018

Figure 6. Dylan M^{c}Farlane demonstrating the benefits of TIF to WA strawberry growers and industry operators, Wanneroo, Western Australia, Feb 2019.

Figure 7. Dylan McFarlane demonstrating TIF and LDPE film effectiveness in retaining fumigant on soil columns, Wandin, Victoria, May 2018.

Figure 8. Project team members with Mr Brendon Hoyle (Ashbern Farms), Stanthorpe, Queensland, Sept 2019.

Outcomes

The outputs produced by this project have resulted in the increased adoption by strawberry growers of practices that reduce charcoal rot. These have included the integrated use of more effective plastic (totally impermeable films (TIFs)), superior chemical fumigants (e.g. Tri-Form® 80 or similar, ethanedinitrile (EDN™ Fumigas)), and improved fumigant application techniques (e.g. shank injection), cultural practices (e.g. removal of dead plants) and farm biosecurity practices. The adoption of these improved farm practices is due to our communication activities (workshops, conferences, articles and field days) with growers and other users (fumigators, agronomists, industry development officers, scientists, chemical and plastics companies). Other users are now recommending to growers treatments identified by this project to manage charcoal rot of strawberry more effectively. For example, fumigators from R&R Fumigation Services Pty Ltd and agronomists from EE Muir & Sons Pty Ltd have both organized separate workshops for strawberry growers in Victoria and South Australia, respectively, centered on the research generated by this project. Moreover, based on our research results, R&R Fumigation Services Pty Ltd have directly recommended that strawberry growers in Western Australia apply fumigants using shank injection rather than drip irrigation. Strawberry Industry Development Officers are also recommending specific farm biosecurity and crop removal practices developed by this project to growers and monitoring their adoption by industry.

Adoption of the following farm practices by Australian strawberry growers, to manage charcoal rot, is a direct result of the research and extension activities conducted by the project team:

Totally impermeable films

Data collected from grower surveys and plastic film manufacturers shows that the use of totally impermeable films (TIFs) by strawberry growers in Victoria, South Australia and Western Australia has increased from 0% in 2017 to >80%, >70% and >35%, respectively (based on area). Similarly, more than 75% of the strawberry growers in Stanthorpe, Queensland, who use soil fumigants have adopted TIF for management of charcoal rot. Results from this project show that the adoption of TIF by the Australian strawberry industry will increase the effectiveness of fumigation by up to 32% (based on fumigant concentrations (ppm) derived from Gastec® detection tubes), reduce the index score of charcoal rot of strawberry by up to 91%, increase strawberry yields by up to 68% and gross revenue from fruit by up to $1.49/plant for the season (Appendix I). Associated benefits to industry are reduced emissions of fumigants to the atmosphere and exposure of applicators to fumigants (Appendix I).

New fumigants

Results from this project showed that the chemical fumigant ethanedinitrile (EDN) controlled charcoal rot by up to 95% compared with untreated soil (i.e. average plant deaths due to charcoal rot was 2.2% for blocks treated with EDN and 47.8% for untreated) (Appendix I). This data assisted the registration of the product for use in the strawberry industry in Australia. This is the first registration of EDN for soil disinfestation anywhere in the world, and the first time a fumigant has been registered for use specifically against *M. phaseolina*. In addition, results from this project showed that products containing low ratios of 1,3-dichloropropene/chloropicrin, such as Tri-Form® 80, also controlled charcoal rot by up to 83% compared with untreated soils (i.e. average plant deaths due to charcoal rot was 7.8% for blocks treated with Tri-Form® 80 and 47.8% for untreated) (Appendix I). In Victoria, 98% of strawberry growers have adopted superior fumigant products (e.g. Tri-Form® 80 or EDN), compared with the previous standard of chloropicrin. Data from chemical suppliers also indicates that significant numbers of strawberry growers in Queensland, South Australia and Western Australia are beginning to adopt these products. Evidence from this project confirms that this practice change will increase control of charcoal rot and marketable yields of strawberry.

Fumigation methods (shank, drip, broadacre, strip)

In 2018, 95% of strawberry growers north of Perth (Wanneroo) in Western Australia applied soil fumigants through drip irrigation. The project team surveyed eight strawberry farms at Wanneroo in February 2019 and found charcoal rot on each property. Results from this project showed that shank injection of specific fumigants increased control of charcoal rot by up to 37% compared with drip applied fumigation (i.e. average plant deaths due to charcoal rot for blocks treated with EDN shank TIF was 5% compared to 8% for EDN drip TIF) (Appendix I). This result supports previous work in Western Australia that fumigants do not reach the shoulders of strawberry beds when applied by drip fumigation (Hort Innovation Project BS12025). Following our communication activities, the adoption of shank application of fumigants by strawberry growers in Western Australia increased in 2020 to represent a third of the industry (based on the production area). Furthermore, the growers that adopted shank

fumigation represent most of Australia's strawberry export production. Evidence from this project demonstrates that this practice change will reduce charcoal rot and increase commercial yields of exported strawberry.

Field experiments showed that broad-acre fumigation under LDPE of the entire paddock reduced deaths due to charcoal rot by 66% compared to strip fumigation of the raised beds under LDPE (i.e. average plant deaths due to charcoal rot was 7% for blocks treated with broadacre fumigation and 20% for blocks treated with strip fumigation) (Appendix I).

Removal of old strawberry crowns

Our research showed that *M. phaseolina* survives in old crowns in the soil from one season to the next, and that the fungus surviving in these crowns rapidly infects new strawberry plants (Appendix I). Based on these results, we have recommended that strawberry growers remove infected strawberry crowns to reduce inoculum pressure in the following crop, as one component of their strategy for managing charcoal rot. Prompted by information delivered at our extension activities, Australian growers started to develop their own farm equipment to remove the previous crop (Figure 9). An article written by Angela Atkinson (Berry industry development officer, VSIDC), and published in the industry magazine "Australian Berry Journal" (Portland strawberries – a charcoal rot success story Bolwarra, Victoria, 2020, Edition 3, pages 64-67), detailed how the adoption of crown removal as part of integrated disease management program helped reduce the amount of *Macrophomina phaseolina* in the soil by 30% and incidence of charcoal rot by 20% on their property. This case study is expected to further drive the innovation and adoption of crop removal techniques by industry. As a non-chemical disease management practice, removal of strawberry crowns is an important option for organic growers and, over time, may reduce conventional growers' reliance on chemical fumigants for control charcoal rot.

Figure 9. Machine designed by strawberry growers to remove old plants and plastic, based on the recommendations from this project.

Farm biosecurity

M. phaseolina is spread from paddock to paddock in infected soil or crop residues. This makes the diligent application of farm biosecurity practices particularly important for managing the spread of the disease. In 2020, results from our surveys showed that 40% of Victorian strawberry growers had adopted at least one new farm biosecurity practice since 2017, to minimize the spread of charcoal rot on their properties (in Monitoring and Evaluation: Page 21, Figure 11). Data showed an association between the increased use of farm biosecurity by growers with lower concentrations of *M. phaseolina* in the soil (in Monitoring and Evaluation: Page 22, Figure 13). We anticipate that the adoption of these improved hygiene practices will reduce the spread of charcoal rot, across and within the Australian strawberry industry. Draslovka Services Pty Ltd (distributor of the EDN fumigant) adopted components of the farm biosecurity plan developed by this project to improve their operating practices.

Crop termination

Crop termination involves killing an old strawberry crop with soil fumigants, such as metham sodium, applied through the irrigation system. Results from trials in this project showed that crop termination followed by pre-

plant fumigation increased fruit yields and revenue in the following crop by $1.29/plant, in comparison to pre-plant fumigation alone. Based on our results, strawberry growers in Queensland and Victoria have started to adopt crop termination for commercial production. The increased adoption of crop termination is expected to increase fruit yields on farms that have a history of charcoal rot.

Crop yields and estimated revenue with adoption of control strategies

Overall, the increased adoption of TIF, new fumigants, cultural practices and farm biosecurity practices by Victorian strawberry growers has improved their control of charcoal rot by 25%, (i.e. from an average of 20% incidence on each property, in 2017, to 15% in 2020) (in Monitoring and Evaluation, page 23). However, disease incidence was reduced by up to 20% on some of the properties in Victoria (in Outcomes: Page 19). From combined figures across Victoria, we estimate the Victorian industry has generated an additional $5M in revenue in 2020 due to their improved management of charcoal rot, compared with 2017. Moreover, based on anecdotal evidence, the adoption of one or more of these farm practices by growers in Western Australia, South Australia and Stanthorpe, Queensland, has significantly improved their management of charcoal rot.

Monitoring and evaluation

This project has delivered outputs (page 11) and outcomes (page 17) well above the line of accountability in the program logic. This is a result of the practicality and applied nature of the research, combined with the strong engagement team members had with growers and other users of the technologies. The project has had great success in generating early adoption of technologies and products by growers, and this has resulted in a measured reduction in disease and increase in productivity in the industry (see section (3) below). Evidence suggests that these productivity gains will continue to increase beyond the life of this project, particularly if industry and Hort Innovation adopt the recommendations for future research to further support treatments identified by this project (page 26). Based on these factors, we recommend that Hort Innovation conduct a detailed evaluation within 1-2 years of this report to calculate the considerable return-on-investment of this project for industry.

At the start of this project, Hort Innovation and the project team set four key evaluation questions:

(1) What is the evidence that the final users have increased their understanding of the disease and its control?

Prior to the phase-out of the soil fumigant methyl bromide in 2006, charcoal rot was an extremely rare disease of strawberry in Australia (Hutton et al., 2013). However, charcoal rot rapidly increased in importance following the adoption of substitute fumigants (Figure 10). The symptoms of the disease are very similar to other wilt and crown rot diseases but result in the widespread death of plants. Very few growers or agronomists recognized the disease or knew what caused it. In 2015, agronomists from Nutrien Ag Solutions® undertook the first survey of the strawberry fruit sector in Victoria on charcoal rot (personal communication). Results showed only 2% of fruit growers were aware of the disease, but *M. phaseolina* was found on plants on 20% of properties surveyed.

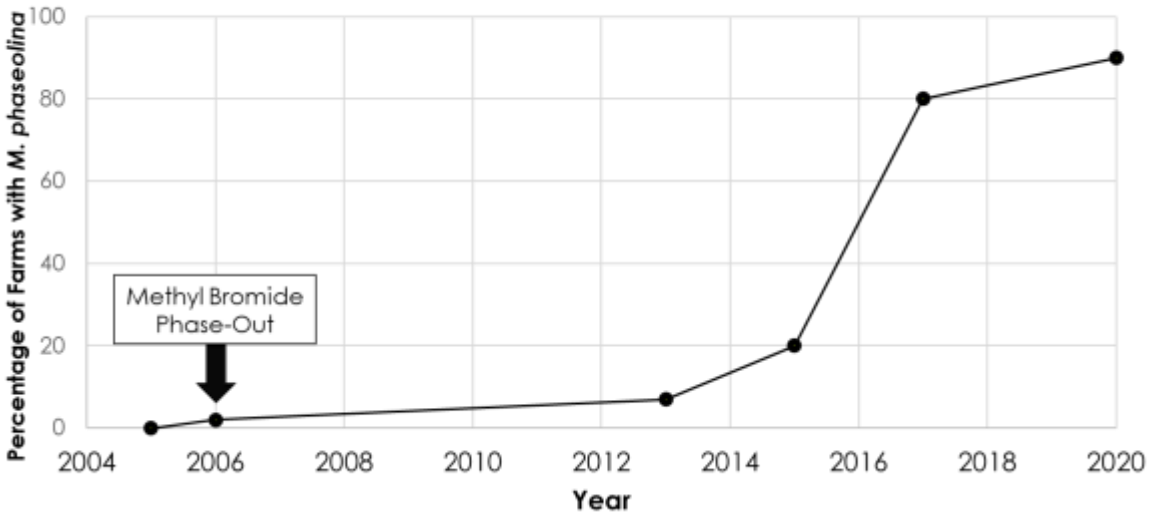

Figure 10. Increase in the percentage of strawberry farms in Victoria infested with the fungus *Macrophomina phaseolina* (cause of charcoal rot) since the phase-out of the soil fumigant methyl bromide.

We conducted an on-farm survey at the start (2017, n = 92) and end of this project (2020, n = 76) with strawberry fruit growers in Victoria (the state where charcoal rot is most prevalent). The survey included: (1) a questionnaire on growers' awareness and management of charcoal rot, (2) measurement of DNA concentrations of *M. phaseolina* in their soil, and (3) assessment of the incidence of charcoal rot in their crops. Results showed that growers' awareness and understanding of the disease, its cause and its management had increased considerably by the end of the project. In 2020, 70% of growers knew that charcoal rot is caused by the fungus *M. phaseolina* infecting strawberry under conducive environmental conditions (compared with 2% in 2015 and 22% in 2017). Furthermore, 78% of growers correctly identified if they had charcoal rot, or not in strawberry plants and soil on their property (based on our previous use of diagnostic tests on their farms).

In 2020, 97% of fruit growers reported they had adopted one or more new practices, since 2017, to manage charcoal rot more effectively (Figure 11). Of the new practices adopted, 70% were those proven effective and

communicated to them by this project. For example, 98% of fruit growers that fumigate had changed to one of the products (Tri-Form®80 or EDN) identified by this project as most effective against charcoal rot. Furthermore, 80% of fruit growers that fumigate had adopted the use of TIFs with fumigants.

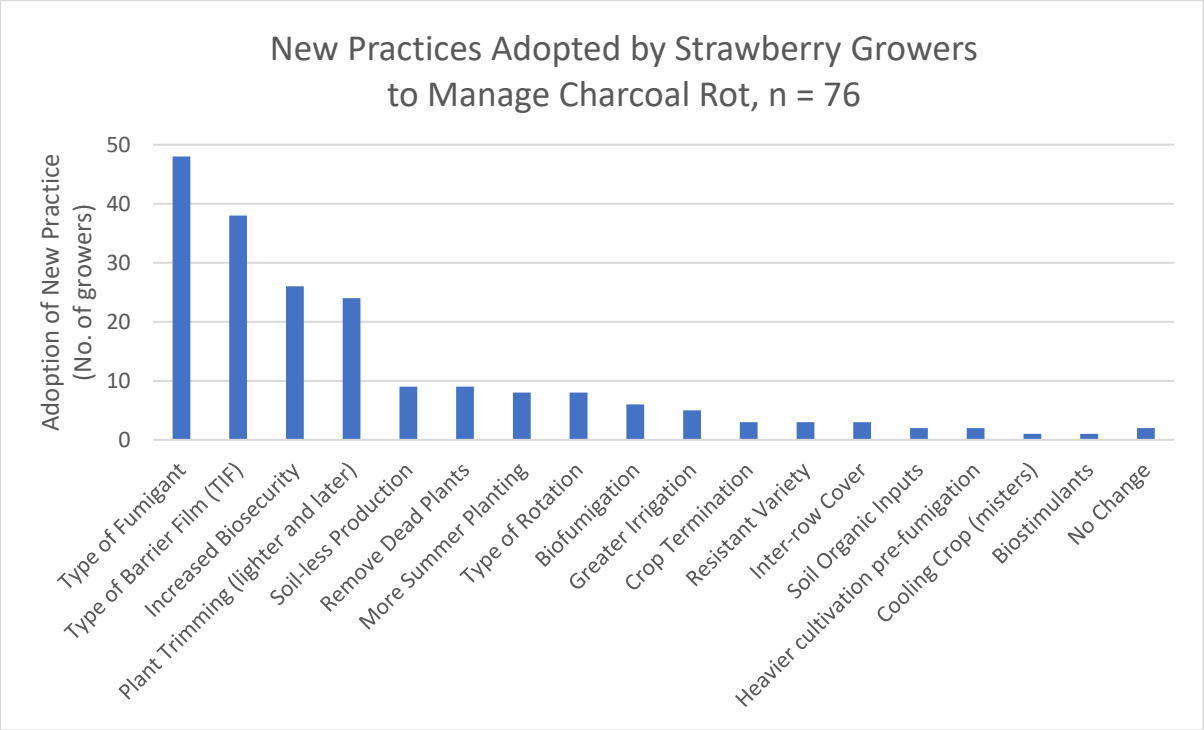

Figure 11. New management practices (since 2017) adopted by strawberry growers in Victoria in 2020 for managing charcoal rot.

(2) To what extent has the project contributed to growers implementing on-farm hygiene measures to prevent the disease spreading?

M. phaseolina is primarily transmitted from field to field in infested soil or residues of infected strawberry plants. This makes farm biosecurity particularly important for managing the spread of the disease. At the start of this project many strawberry growers were complacent about farm biosecurity. This attitude probably developed from growers' previous reliance on methyl bromide, which controlled all soil-borne pests very effectively, eliminating the need to employ additional management practices.

In 2018, this project conducted a series of presentations and demonstrations on how growers could implement practices of farm biosecurity to better manage the spread of charcoal rot. Many growers expressed a reluctance in adopting these measures, due to cost or inconvenience. However, results from phone interviews with grower attendees three month after the events showed 75% adopted at least one method, such as training their staff on their biosecurity expectations (McFarlane et al., 2019b).

By 2020, results from the on-farm survey (see above) showed 100% of growers used at least one of the eleven farm biosecurity practices discussed at project events (Figure 12).

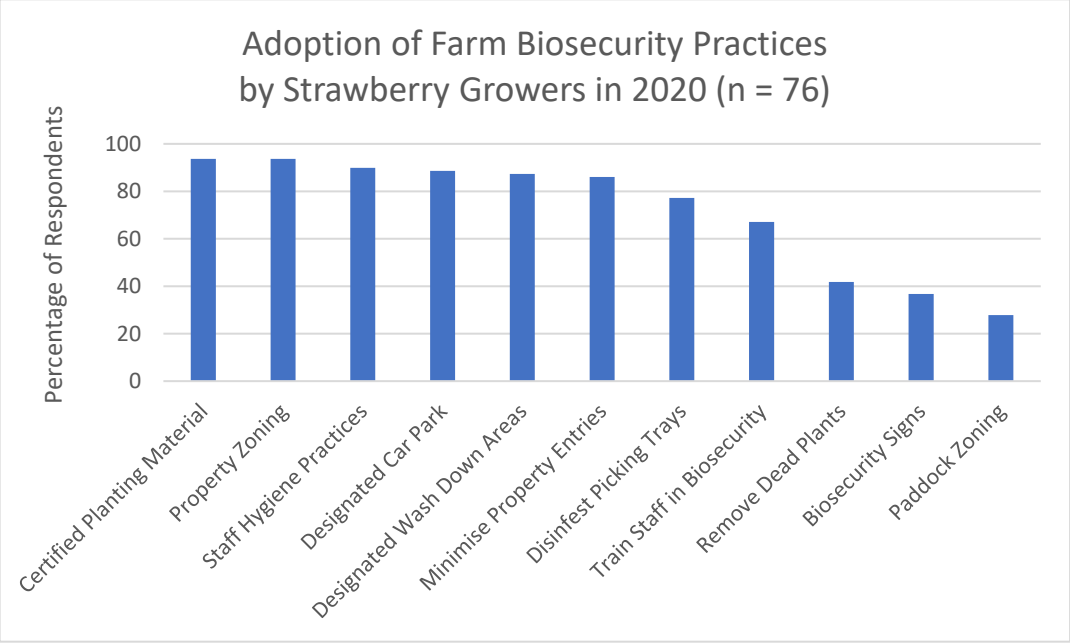

Figure 12. Use of farm biosecurity measures by strawberry growers in Victoria to assist in managing the spread of charcoal rot within and between farms.

Results also showed there was a strong association between increased adoption of biosecurity practices and lower concentrations of *M. phaseolina* in soil on strawberry farms (Figure 13). Despite this, 60% of growers still did not think that improving biosecurity practices was important in managing charcoal rot. Furthermore, many growers expressed frustration with utility providers (e.g. water, electricity) and labour force contractors in not following their biosecurity requirements on their farms. This shows there are strong opportunities to increase education and awareness in the importance of farm biosecurity with these groups, as well as strawberry growers, in future projects or extension programs.

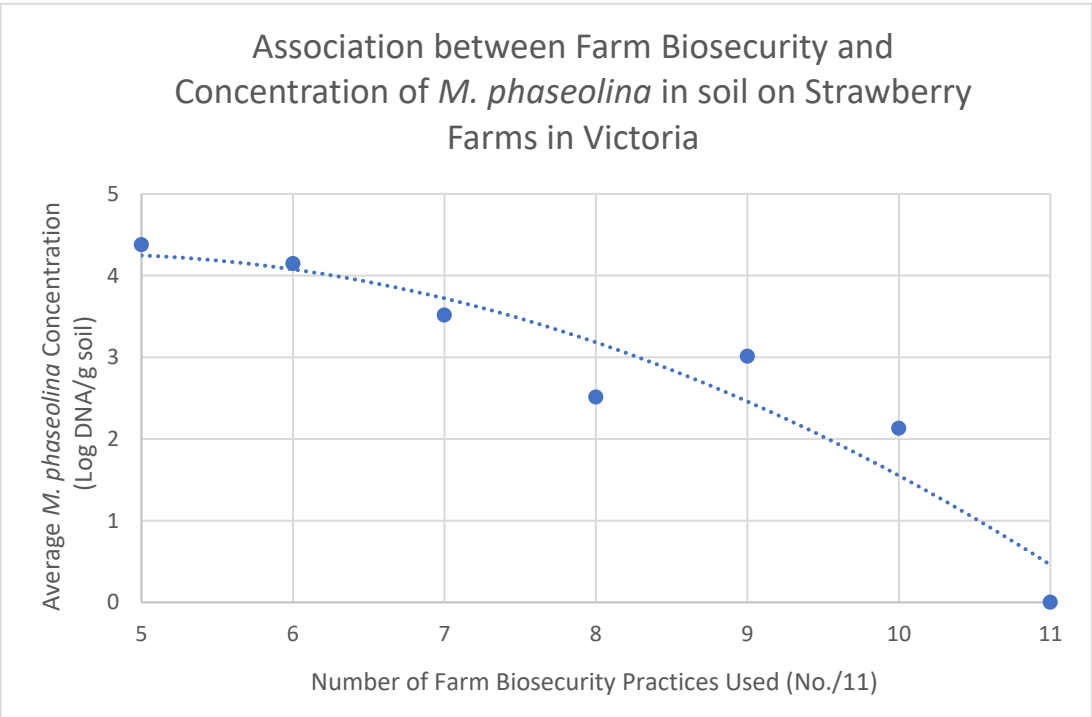

Figure 13. The relationship between the number of farm biosecurity practices used by growers and the concentration of *Macrophomina phaseolina* (the cause of charcoal rot) in soil and on strawberry fruit farms in Victoria (n = 76). Note: no growers used fewer than five farm biosecurity practices on their farms.

(3) Did the project deliver planned outputs and outcomes within budget and timeframes?

On average, this project delivered approximately one communication output every fortnight across the life of the project (in Outputs: page 11). All contracted outputs were delivered on time and on budget.

At the start of this project, we developed a program logic linking planned outputs with intended outcomes from our work (Figure 14). Next- and final-users of the outputs from the project included: strawberry fruit and nursery growers, chemical and plastic mulch manufacturers and distributors, agronomists, fumigators, IDOs and extension officers, other scientists and Hort Innovation. Evidence shows that progress against the program logic is tracking well above the line of accountability for the project. For example, chemical companies have significantly shifted their attitudes as a result of this project because they now recognize the importance of evaluating and including *M. phaseolina* on the product label for strawberry (previous to this project no fumigant products included *M. phaseolina* on the label). Similarly, the attitudes of fumigators and plastics manufacturers have changed because they are now only selling (Tri-Form®80 or similar, EDN) or promoting (TIF) the use of products identified as effective against *M. phaseolina* by this project. Furthermore, they have started running their own grower events and producing newsletter advertorials using data from this project.

The outputs from this project and the attitudinal shifts they created in other users have assisted in driving practice changes by strawberry growers towards products and practices that improve control of charcoal rot (Figures 11 & 12, and Outcomes pages 11-16). These practice changes are already delivering intermediate outcomes (e.g. increased farm productivity) above the line of accountability identified in the program logic. For example, results from the on-farm surveys showed that the incidence of charcoal rot in strawberry fruit sector in Victoria fell from 20% in 2017 to 15% in 2020. This reduction in disease was associated with the partial adoption of TIFs and superior fumigants. Growers and plastics manufacturers showed a preference to use their existing stocks of LDPE before fully adopting TIF, delaying the initial adoption of TIF. Based on the timing of disease and its effect on strawberry yield in Victoria, we estimate the 5% reduction in disease incidence increased productivity by 2.5%, worth $5 M to the sector in 2020 ($200 M p.a. GVP in Victoria, FreshLogic). Based on evidence from our trials showing TIFs and improved fumigants reduce the incidence of charcoal rot by an average of 68% and the projected further adoption of these technologies, we estimate the incidence of charcoal rot will fall to 10% in the sector by 2022. Based on 2020 figures, this would increase productivity by 5%, worth $10 M p.a. to the sector.

Increased farm productivity is an important outcome from this project because it aligns with the industry's Strategic Investment Plan and assists it towards its goal of a 10% increase in marketable yield from 2017 to 2021.

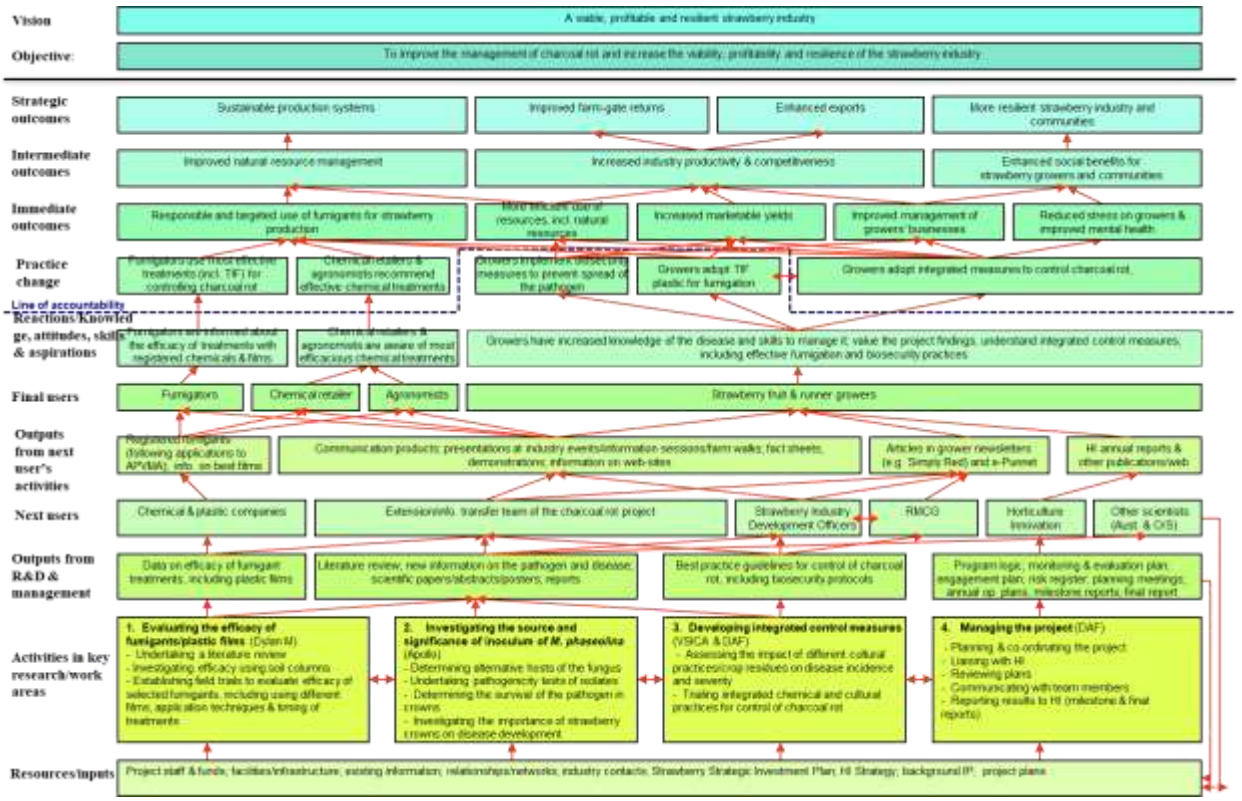

Figure 14. Program logic for 'BS15005 Improved management of charcoal rot' (2018) linking project outputs with desired outcomes for the strawberry industry and other users.

(4) How well were industry stakeholders engaged in the project and did the project meet the needs of industry end-users?

We estimate that we have reached 75% of strawberry growers across Australia with our communication outputs from this project (see pages 11-16). In addition to these tangible outputs, one of the most effective extension tools in this project was communicating directly with growers during visits to their farms. The project team visited 132 strawberry farms across every state of Australia, and this built strong connections with growers. It also allowed the team to deliver tailored information that was highly relevant at a regional level. The following growers' comment was typical of the qualitative feedback we received from them after the visits:

> 'THANK YOU for helping progress the industry with really proactive measures. We learnt from your visit last time and we learnt more today. We will be better farmers for it.'
>
> Nina Meiers (Barjarg, Victoria)

The comprehensive and practical nature of the science in this project was reflected by the unsolicited feedback we received from overseas experts on charcoal rot of strawberry. At a grower workshop, visiting scientist Dr John Washington, Global R&D Director TriCal group USA (largest fumigation company in the world) said:

> 'One thing that I learned from the group's research that really impressed me were the number and variety of research trials that have been undertaken. I just want to share my enthusiasm for what I've seen and also my optimism that there will be an economic solution for growers to managing this disease.'

Similarly, Steven Koike an extension scientist from University of California and the first researcher to extensively describe charcoal rot of strawberry commented:

> '[This project is]… the most comprehensive program on charcoal rot of strawberry anywhere is the world'.

The quality of this project in meeting the needs of the strawberry industry was also reflected in comments from Dr Brenda Kranz (R&D Manager, Hort Innovation):

> 'coming to the recent project meeting really helped me to see the context of the project activities and how they are delivering significant benefits to industry'.

Lessons from the Project

We regard the following factors as important lessons for future research in the strawberry industry on charcoal rot or other pests and diseases:

1) Pick strawberry fruit and determine yields for the entire season in research trials.

Trials in the strawberry fruit industry are costly because berries require picking 2-3 times per week for up to 14 months to determine yield. To reduce costs, some researchers have picked fruit for short periods at different points through the season to estimate yield. This approach does not work for trials on charcoal rot because of the uncertain timing and rapid development of the disease. Determining full yields through the season was also vital for calculating the economics of different treatments because the price of fruit varies considerably through the year. Calculating the economics of different management practices was a key factor in driving high rates of adoption by growers during this project, especially considering that many treatments (e.g. TIF) were more costly than standard practices.

2) Quantify the extent of the problem (disease) at the start of the project.

The project team met with more than 90% of the strawberry growers from Victoria and surveyed their properties in 2017 and 2020 for charcoal rot. The survey in the first year of the project (2017) was important in setting a baseline for charcoal rot incidence and severity, grower awareness of the disease and grower management practices, across the state. The baseline data is essential for being able to demonstrate change over time with a survey at the end of a project. Yet it can be difficult to engage the broader grower population for the initial survey as they are often not conversant with the topic or project. The survey delivery method can have a significant impact on the response rate and hence volume of (baseline) data generated. The initial survey in Queensland was online and received a very poor participation rate; a stark contrast to the >90% response rate to the in-person survey in Victoria. The survey in 2020, which assessed the same parameters as in the initial survey, evaluated how the industry had changed over the course of the project (i.e. 2017-2020).

3) Well executed industry survey can transform behaviour around information sharing

In Victoria, both surveys became powerful communication tools that promoted best practices amongst growers and led to behavior shift to more open information sharing on a sensitive topic between growers. An absence of information sharing between growers within an industry can be common where a topic is particularly sensitive (e.g. disease severity) or perceived to impart a commercial advantage. The in-person surveys provided growers with the confidence to disclose their individual information. Presentation of the collated results at industry events led to a change in attitude with growers becoming more open in sharing information. The volume of data generated from the high response rate can assist industry in strategic decision-making.

4) Frequent industry engagement and information delivery to enhance adoption.

Three successful techniques for greater industry adoption identified during this project were:

i. Interaction with the broader grower population (e.g. baseline survey) early in the project followed by frequent delivery of information updates throughout the project life led to growers being highly responsive to adoption of improved practices developed in the project.
ii. Service providers can be a valuable additional pathway for delivery of information where a close grower-service provider relationship exists, as was evident in Victoria and conversely absent in Queensland.
iii. Participatory activities (e.g. soil column demonstrations) are very well received by growers and hence are an effective method for reinforcing key messages delivered in oral presentations or articles.

Recommendations

Hort Innovation

The progress to date still leaves several gaps in knowledge of the biology of *M phaseolina* and how best to manage charcoal rot. The project successfully concentrated on identifying alternative chemical fumigants to address the immediate need of growers in their control of charcoal rot. Research to develop a broader set of control options is required to sustain industry into the future, provide effective disease controls for all industry sectors (conventional, organic, nursery), and build a greater level of precision and flexibility in managing charcoal rot. Areas of research with the greatest benefit to growers include:

- A reliable diagnostic tool for predicting the disease risk on farm and informing the most cost-effective choice of treatments.
- Non-chemical soil treatments for reducing the amount of the pathogen before planting.
- New treatments with the potential for managing charcoal rot outbreaks during the growing season.
- Quantifying the soil and environmental conditions that trigger the disease.
- Integrating the use of tolerant varieties with other treatments for more effective management of charcoal rot.

Several diagnostic tests have been developed in Australia and overseas for monitoring the level of *M. phaseolina* in soil. Currently, however, it is not possible to predict the risk of disease using these tests. This is because results of *M. phaseolina* concentration in soil have not been correlated with the amount of disease to develop in a crop. The ability to predict the level of disease risk would add precision and flexibility in managing charcoal rot, with the bonus of a potential to reduce costs.

The Australian strawberry industry is heavily reliant upon chemical fumigants for the control of charcoal rot. However, chemical fumigants do not provide complete control of the disease, and are under constant threat of registration review and withdrawal, due to concerns over detrimental effects on human and environmental health.

Studies overseas have indicated several alternative treatments that reduce the pathogen in the soil to varying degrees, including: biofumigation with brassica crops, anaerobic soil disinfestation, high soil temperature by microwave or steam treatment, repeated removal of plant debris for the depletion of pathogen inoculum over the medium to long term, and crop rotation. The suitability and effectiveness of these techniques in local strawberry production systems has yet to be proven.

Non-chemical treatments give industry the opportunity to reduce chemical usage and provide control options for organic growers. Non-chemical treatments may complement, offset or replace the need for chemical fumigants for controlling charcoal rot.

All practices currently available for managing charcoal rot are confined to the pre-plant period. Developing strategic treatments and technologies for managing the disease during the season will require research to develop an understanding of the conditions (soil, environment, plant health) that trigger infection and foster disease development.

Although immunity in strawberry to charcoal rot has not been found, research indicates varieties differ in their tolerance of the disease (BS12021 National Strawberry Varietal Improvement Programme). Quantifying the performance of tolerant varieties when grown in the field with the best practices for controlling charcoal rot, would confirm the effectiveness of such a multi-faceted strategy.

Strawberry Fruit Growers

- Establish a farm biosecurity plan to protect against the spread of charcoal rot within and between farms. Train farm staff in hygiene practices for use in daily operations.
- Use totally impermeable films (TIFs) when applying chemical fumigants, in preference to low-density polyethylene films (LDPE), for greater disease control and increased fruit yields.
- EDN™ Fumigas, Tri-Form® 80 and similar formulations of chloropicrin and 1,3-dichloropropene, are the most effective chemical fumigants currently available for control of charcoal rot.
- Apply soil fumigants under optimal conditions (see product label) to maximize their effect, paying particular attention to:
    - Read the product label before fumigating to ensure that the weather conditions are appropriate for fumigation.

    - Aerate soil before fumigation by rotary-hoeing and ripping. Ensure that the soil structure is uniform and free of clods to maximize movement of fumigant gases through the soil profile.
    - Check the soil temperature at 15cm depth early on the day of fumigation.
    - Ensure the soil is at 70% field capacity for 7 days before and on the day of fumigation.
    - Consider a broad-acre application of fumigants when treating paddocks that have previously had a high level of charcoal rot.
    - Conduct a soil test for the presence of fumigant residues before planting a strawberry crop (e.g. lettuce seed test).
- Apply chemical fumigants by shank inject fumigants rather than applying them through drip-tape, if both options are provided on the label.
- Remove infected crowns when possible and avoid incorporating back in the ground to reduce risk of pathogen population increasing.
- As symptoms of charcoal rot are similar to those caused by other soil-borne pathogens, it is recommended that infected plants are submitted to a diagnostic laboratory to confirm the causal agent, and enable the appropriate management option to be applied.
- Avoid planting sorghum as cover-crop and alternative hosts of *M. phaseolina* (e.g. watermelon) in strawberry cultivation blocks with a history of charcoal rot.

Strawberry Nursery Growers

Charcoal rot rapidly became established in the strawberry fruit sector in Australia following the phase-out of the soil fumigant methyl bromide. To date, researchers and inspectors have not detected *M. phaseolina* in soils on strawberry nurseries, or in certified runners. However, charcoal rot occurs on strawberry fruit farms close to strawberry nurseries in Queensland and Victoria. We recommend that strawberry nursery growers consider the following factors to minimize the risk of charcoal rot spreading to their nurseries:

- Maintain high levels of farm biosecurity to minimize the risk of infested soil entering production areas on your nursery.
- Monitor on-going research programs aimed at developing alternatives to methyl bromide for runner production.
- Continue the use of methyl bromide, where allowed under a critical use nomination, because this fumigant is proven to eradicate charcoal rot. Continue the practice of broad-acre application of fumigants because this method treats all soil in the production zones.
- Use of TIFs with soil fumigants, wherever possible, to maximize effectiveness against soil-borne pathogens and pests, and to minimize emissions of fumigants to the atmosphere.
- Familiarize yourself with the symptoms of charcoal rot and send plants with wilting symptoms to a diagnostic service to confirm cause.
- Consider the role of soil-less systems for production of plug plants. These systems avoid the need for soil and therefore can reduce the risk of soil-borne diseases, like charcoal rot. Be aware these methods rely on strict practices of farm biosecurity to prevent contamination by infested soil or infected plant material.

Australian Government

Strawberry industries around the world previously relied on the use of the soil fumigant methyl bromide for controlling soil-borne pathogens and pests. However, research implicated emissions of methyl bromide in the degradation of stratospheric ozone. Consequently, the United Nations (UN) listed methyl bromide for withdrawal under the *Montreal Protocol*. The strawberry fruit sector in Australia was amongst the first worldwide to phase-out methyl bromide. Following the withdrawal of methyl bromide, however, the incidence of charcoal rot in strawberry increased in Australia; hence, the importance to develop integrated strategies to manage the disease also increased.

There are several exemptions from the phase-out of methyl bromide within the *Montreal Protocol* (quarantine and pre-shipment applications (QPS, a non-regulated use), critical-use, and emergency-use). Each exemption requires that applicants meet strict regulations and conditions. Currently, there are several exemptions for methyl bromide granted to countries (Argentina, Australia – nursery runner production only, USA) that assist in the management of charcoal rot of strawberry (e.g. 370 tonnes to strawberry nurseries in the USA under QPS). In addition, government in the USA is investigating the possibility of emergency uses of significant quantities of methyl bromide for

controlling charcoal rot in their strawberry fruit sector (Ruckert, 2019). The findings of the investigation are due for release in late 2020 / early 2021 (Heinzman, 2020).

If strawberry fruit industries in other countries receive significant exemptions to use methyl bromide for controlling charcoal rot, then the Australian industry may likewise pursue a similar exemption. We recommend that the Australian government through the Department Agriculture, Water and Environment maintain an awareness of the situation, and actively monitor any exemptions for methyl bromide for managing charcoal rot that may occur in other countries.

Refereed scientific publications

Scientific publication

McFarlane, D. J., Mattner, S. W., Gomez, A. O. & Oag, D. R. 2020. The impact of soil fumigants and planting material on charcoal rot of strawberry in Australia. *Acta Horticulturae* (in-press)

Conference proceedings

Gomez, A. O., McFarlane, D. M., Mattner, S. W., Greenhalgh, F. C. & Oag, D. R. 2018. Survival of *Macrophomina phaseolina* in strawberry crop residue. *10th Australasian Soilborne Diseases Symposium*, Adelaide, Australia, September 2018, Poster 3, 28.

Gomez, A. O., McFarlane, D. M., Mattner, S. W. & Oag, D. R. 2019. Infected crop debris is an inoculum source of *Macrophomina phaseolina* in strawberry. *Methyl Bromide Alternatives Outreach Fumigation Alternatives for Production, Storage and Trade Conference,* 56.

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Intellectual property, commercialization and confidentiality

No project IP, project outputs, commercialization or confidentiality issues to report.

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We are forever grateful of the patience and cooperation of the multiple growers who hosted one of the field experiments within the project. The field experiments were essential for confirming the effectiveness of the improved management practices in a commercial production system.

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Appendices

Appendix I. Improved management of charcoal rot of strawberry: Research results.

Appendix I. Improved management of charcoal rot of strawberry: Research results.

(BS15005)

Table of Contents

Abbreviations

Table 1. List of fumigant products and their active ingredients.

Product name	Chemical actives
EDN™ Fumigas	100% ethanedinitrile
Tri-From® 80	80% chloropicrin: 20% 1, 3-dichloropropene
Agrocelhone® FE	59.5% chloropicrin: 39.5% 1, 3-dichloropropene
Basamid® Granular	98% dazomet
Metham sodium soil fumigant	metham sodium 423g/L
Paladin®	98.8% dimethyl disulphide
Pic Plus® Fumigant	85.5% chloropicrin
Methyl bromide recaptured from quarantine applications on carbon	4-8% methyl bromide
MiPic®	98% methyl iodide
DOMINUS®	96.3% Allyl isothiocyanate

Table 2. List of abbreviations.

Abbreviation	Description
EDN	EDN™ Fumigas: Soil fumigant
TF80	Tri-From® 80: Soil fumigant
AGRO	Agrocelhone® FE: Soil Fumigant
DMDS	Dimethyl disulphide: Soil Fumigant
PIC	Pic Plus®: Soil fumigant
MB(Q)	Methyl bromide from quarantine applications: recaptured on activated charcoal. The charcoal is spread across the soil surface and incorporated to a depth of 25 cm with a rotary hoe. This is a different product and application method to shank-injected formulations of methyl bromide, which were phased-out under the Montreal Protocol. Quarantine sources of methyl bromide are not regulated under the Montreal Protocol and the recaptured product may be recycled into soil.
TIF	Totally impermeable film: Plastic tarp used to cover soil after fumigation. This tarp contains a layer of ethylene vinyl alcohol, which makes it impermeable to the movement of fumigants.
LDPE	Low density polyethylene: Plastic tarp used to cover soil after fumigation. This is the 'standard' tarp used by most strawberry growers in Australia, but is permeable to the movement of fumigants.
Strip	Strip/bed fumigation: Only the raised soil beds are fumigated, not the inter-rows between them.
Broad	Broadacre fumigation: The entire block is fumigated, including the inter-rows between the raised soil beds.
Shank	Shank injected fumigation: The fumigant is injected into the soil to a depth of 15 cm, through tynes spaced 30-50 cm apart using a mechanical rig that is trawled behind a tractor.
Drip	Drip fumigation: Emulsified forms of fumigant are mixed with water (ratios are described on fumigant labels) and applied to beds covered with plastic mulch through the irrigation system (drip/trickle tape).

1. Summary

Charcoal rot has developed into a major disease in the Australian strawberry industry and growers were in need of new practices that would enable improved control of the disease. Research showed that fumigation with either Tri-Form® 80 or EDN™ Fumigas reduced charcoal rot of strawberry by 71% compared with the untreated controls. Totally impermeable film (TIF) retains chemical fumigant in the soil at higher concentrations and for longer than low-density polyethylene. The additional cost of TIF plastic is outweighed by the additional gross income ($1.49/plant) over the season. The pathogen was shown to survive in buried crowns from one season to the next in both temperate and subtropical production regions in Australia. Although a major source of inoculum, removing crop debris did not lead to any significant difference in plant death or yield in a field experiment of one season. The potential to reduce pathogen inoculum from the cumulative effect of repeated removal of infected crop debris will require a longer-term study. Likewise, further research on biofumigants and non-chemical fumigants will add to the control options available to growers for managing charcoal rot, whilst the evaluation of diagnostic tests inform growers when selecting the most cost-effective treatment for their disease pressure.

2. Introduction

Charcoal rot is a major disease of strawberry capable of causing devastating plant deaths and a substantial financial impact through lost income. Charcoal rot is a soil borne disease caused by the fungus *Macrophomina phaseolina* and has been found in strawberries in all states of Australia. The disease became increasingly prevalent from 2005 and the withdrawal of methyl bromide, leading to an urgent need within the strawberry fruit industry for substitute soil fumigants. In 2017, DAF and VSICA commenced a three-year research project (BS15005), funded through Hort Innovation, to develop new practices, including farm biosecurity, chemical and cultural options, to enable strawberry growers to improve the control of charcoal rot.

Practices common throughout the strawberry industry at the start of the project were fumigation with chloropicrin, low density polyethylene (LDPE) plastic film, and returning strawberry plant debris to the soil at the end of the season. Growing a cover crop of sorghum, a susceptible host of *M phaseolina*, between strawberry seasons was practiced in regions where conditions permitted.

Soil fumigants are chemicals that act as gases against soil-borne pathogens, weeds and pests. At the outset of the project the soil fumigants and disinfestation practices available did not adequately manage the disease. The major research activity was to evaluate fumigant options, including combinations, mixtures, application techniques and new chemistries, to enable strawberry growers to manage charcoal rot more effectively.

Returning infected crop debris to the soil at the end of the season will potentially perpetuate the pathogen in the soil and may increase inoculum levels each year. Further, how long the pathogen survives in buried strawberry crop debris was not known. Equally, *M. phaseolina* has a wide host range including many weed species and crop plants that may be found on strawberry farms. The second focus in the research was to determine the role of infected strawberry crop debris and other sources of inoculum in disease outbreaks in the paddock, and identify alternative hosts of *M. phaseolina* within strawberry production systems.

The objective was to develop an integrated strategy for the improved management of charcoal rot in strawberry.

3. Methods

3.1. Soil columns

3.1.1. Fumigation

Columns were constructed using high-density polyethylene (HDPE) to reduce their absorption of the fumigants and to retain them for the duration of the experiment. The cylindrical columns were 40cm tall and had a diameter of 28cm. Four bulk-head fittings were attached to the columns at depths of 5cm, 15cm, 25cm and 35cm, from the top, to provide gas sampling ports that were accessible on the exterior surface of the columns. Columns were filled and packed with soil to a similar bulk density as in the field. Once the columns were filled, a cylindrical brass probe (L: 26cm, D: 4mm) was inserted into each of the sampling ports in the soil and tightened with fittings to reduce gas leaks. The exterior side of the probes were temporarily closed with HDPE tubes that could be removed for sampling. The soil (silty clay: 26% silt, 41% clay, 33% sand) used to fill each column was obtained from a commercial strawberry farm with known concentrations of *M. phaseolina* DNA.

Fumigation of the columns simulated shank injection in the field (see section 3.2.1.). In this procedure, fumigants were manually injected into the soil and covered with a plastic mulch. Fumigant concentrations were measured with colour metric detection tubes (Gastec®) and/or a MiniRae device. Fumigant concentrations were recorded in soil in the columns at 2, 24, 72 and 168hrs after treatment at depths of 5cm and 25cm.

3.1.2. Pathogen viability in crowns

Prior to fumigation, muslin bags containing five infected crowns were placed into each column at soil depths of 10cm and 25cm. Two weeks post fumigation, the muslin bags were recovered, and the crowns removed. The crowns were tested for the presence of *M. phaseolina* and the percentage of infected crowns was recorded (see section 3.2.2.).

3.1.3. *M. phaseolina* concentrations in soil

Soil samples (500g) were taken at depths of 0-5cm and 25-30cm, 2 weeks after fumigation. Soil samples were analysed by SARDI to determine the amount of *M. phaseolina* DNA present in each sample.

3.2. Field experiments

3.2.1. Fumigation

A study by Mattner *et al.* (2018) identified several properties with relatively high concentrations of *M. phaseolina* DNA in their soil and high severity and incidence levels of charcoal rot of strawberry. Several of these commercial properties were used to host field experiments for the project. The quantity of *M. phaseolina* DNA in the soil, across each site, was determined prior to the experiments with qPCR analyses, conducted at the South Australian Research and Development Institute (SARDI).

Fumigants were applied to soils using several methods, including shank fumigation, drip fumigation and incorporation via rotary hoe. Shank fumigation is the process of injecting fumigants into soil to a depth of 15 cm, through tynes spaced 30-50 cm apart using a mechanical rig that is trawled behind a tractor. Drip fumigation is the process of mixing emulsified forms of fumigants with water (ratios are described on fumigant labels) and applied to beds covered with plastic mulch through the irrigation system (drip/trickle tape). Some fumigants were spread across the surface of the soil followed by rotary hoeing to evenly distribute the product.

Soils were covered with a plastic mulch prior to (drip fumigation) or shortly after treatment (shank injection) to seal the fumigants into the soil for a longer period. The 'standard' film used across the industry at the start of this project was low-density polyethylene film (LDPE). Totally impermeable films (TIFs) are also made from polyethylene, but additionally contain a layer of ethylene vinyl alcohol. The ethyl vinyl alcohol layer in TIF makes the film impermeable to the movement of fumigants, whereas standard films to not completely seal fumigants in soil. We compared the use of fumigants sealed with LDPE or TIF in our experiments.

We measured fumigant concentrations in the soil using colour metric detection tubes (Gastec®). In this procedure, air is drawn through a probe that has been inserted into the soil, and the colour metric tube to detect chemical concentrations. Fumigant concentrations were recorded in soil at a depth of 10cm at 2, 24, 72 and 168hrs after treatment.

3.2.2. Pathogen viability in crowns

Strawberry crowns were collected from plants showing symptoms of disease at field sites that had a history of charcoal rot. A sub-sample of the collected crowns from each site (n = 10) were destructively tested in the laboratory to confirm that they were infected with *M. phaseolina*. One day prior to fumigation, muslin bags containing five infected crowns were buried in the centre of each plot at a depth of 25 cm, on the shoulder of the bed. Two weeks post fumigation, the muslin bags were recovered, and the crowns removed.

The crowns were tested for the presence of *M. phaseolina* and the percentage of infected crowns was recorded. To test for the presence of *M. phaseolina*, crowns were broken apart, under aseptic conditions, to expose their xylem tissue. Four pieces of xylem tissue were removed from each crown, using secateurs, surface sterilised (1% NaOCl, 10 sec) and placed onto a separate plate containing potato dextrose agar (PDA) media (half strength). The plates were incubated at 22˚C and 30% humidity for seven days, and fungi growing

from the crowns onto the PDA were morphologically assessed under a compound microscope to confirm the presence or absence of *M. phaseolina*.

3.2.3. *M. phaseolina* concentrations in soil

Soil samples (500g) were taken from each plot at several time points, post fumigation. Soil samples were taken from depths of 0-10cm, with the use of a hand spade (composites of 10). Soil samples were analysed by SARDI to determine the amount of *M. phaseolina* DNA present in each sample.

3.2.4. Charcoal rot incidence and severity

The plants were regularly assessed for charcoal rot incidence and severity using the scale described by Mattner *et al*. (2018). The charcoal rot severity and incidence scores were converted into decline/death indices (%DI) based on the method described by Fang *et al*. (2013), where:

$$\%DI = \{[(a \times 0) + (b \times 1) + (c \times 2) + (d \times 3) + (e \times 4) + (f \times 5)] \times 100\} / [(a + b + c + d + e + f) \times 5]$$

where a, b, c, d, e and f are the number of plants with a score of 0, 1, 2, 3, 4 and 5, respectively. Diseased plants were defined as plants with symptoms of charcoal rot, including wilting, crown rot and/or death. A sub-sample of the diseased plants were collected from each plot, at the end of each experiment, to confirm the presence of *M. phaseolina*, using laboratory analyses (see section 3.2.2.).

3.2.5. Weed assessments

Weed counts, based on the number of weeds in each planting hole, were conducted during the experiments at several time points. Sampling dates were selected based on the weeding schedule at each site. The number and identify of weeds emerging in each planting hole per plot was determined via manual count. The data sets for each sampling date were analysed together.

3.2.6. Fruit yields and revenue

Strawberries were picked and weighed from plants in each plot, two to three days per week during the peak season (Summer) and once a week when production was low (Autumn). The total fruit weight and number of strawberries produced was recorded. Moreover, the number of marketable fruit (based on fruit quality), their total weight, average size and their generated revenues were also measured. Fruit quality was based on visual inspections using commercial guidelines. The marketable fruit revenues were based on, weekly, Melbourne wholesale prices (Freshlogic, Hawthorne, Victoria). Results were expressed on a "per plant" basis.

3.3. Statistical analyses

All statistical analyses were performed in Genstat v. 18th Ed. (VSN International) using ANOVAs (general function or unbalanced functions). Homogeneity of variance was determined by examining plots of fitted values versus residuals, while histograms of residuals were examined for normality of distribution. Where variance was heterogeneous across treatments, appropriate data transformations (e.g. log transformations) were made to restore homogeneity. Fisher's least significant difference (LSD) test was used to identify significant differences between treatment means. The level of significance used was $P \leq 0.05$.

4. Soil Column Experiments

4.1. Soil column experiment 1: Effectiveness of totally impermeable films (TIFs) for retaining fumigants in soil

Completed: 18/12/2017.

4.1.1. Aims

To determine if totally impermeable film (TIF) would retain higher concentrations of the fumigant Tri-Form® 80 in soil, compared with low density polyethylene (LDPE) film.

List of treatments

- Tri-Form® 80 (400 kg/ha) under TIF.
- Tri-Form® 80 (400 kg/ha) under LDPE.
- Untreated under TIF.

- Untreated under LDPE.

4.1.2. Results

4.1.2.1. Fumigant concentrations

In columns treated with Tri-Form® 80, there was no significant difference in the concentrations of 1,3-dichloropropene ($p = 0.124$) or chloropicrin ($p = 0.452$) between the two sampling depths (Figures 1 and 2). Sealing columns with TIF retained significantly higher concentrations of 1,3-dichloropropene in the soil (up to 3 times greater), compared with sealing with LDPE ($p < 0.001$).

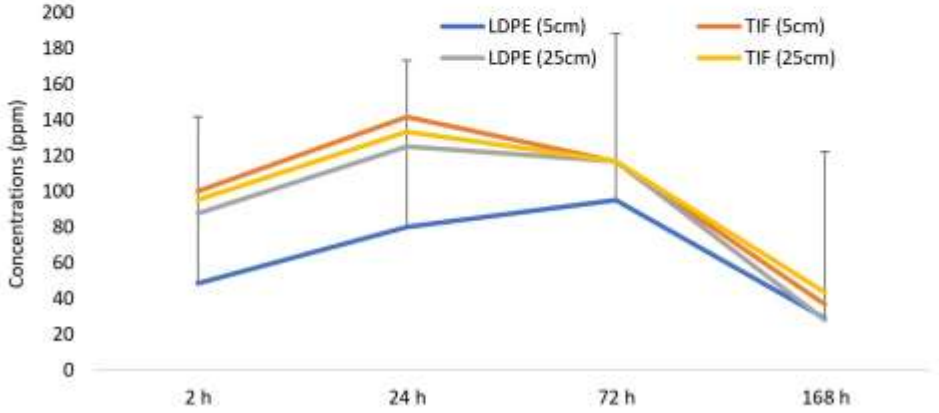

Figure 1. Concentrations of chloropicrin inside soil columns treated with Tri-Form® 80, post fumigation. Bars represent LSDs where $p = 0.05$.

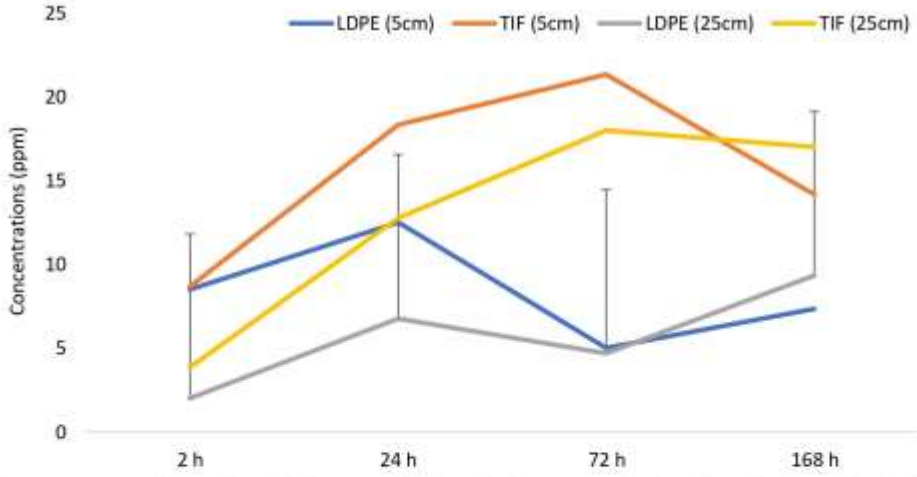

Figure 2. Concentrations of 1,3-dichloroproene inside soil columns treated with Tri-Form® 80, post fumigation. Bars represent LSDs where $p = 0.05$.

4.1.2.2. Pathogen viability in crowns

Fumigation significantly reduced the viability of *M. phaseolina* in crowns in the compared with the untreated controls (Figure 3). Soil depth ($p = 0.75$) and plastic film ($p = 0.526$) did not significantly affect the recovery of *M. phaseolina* from buried crowns.

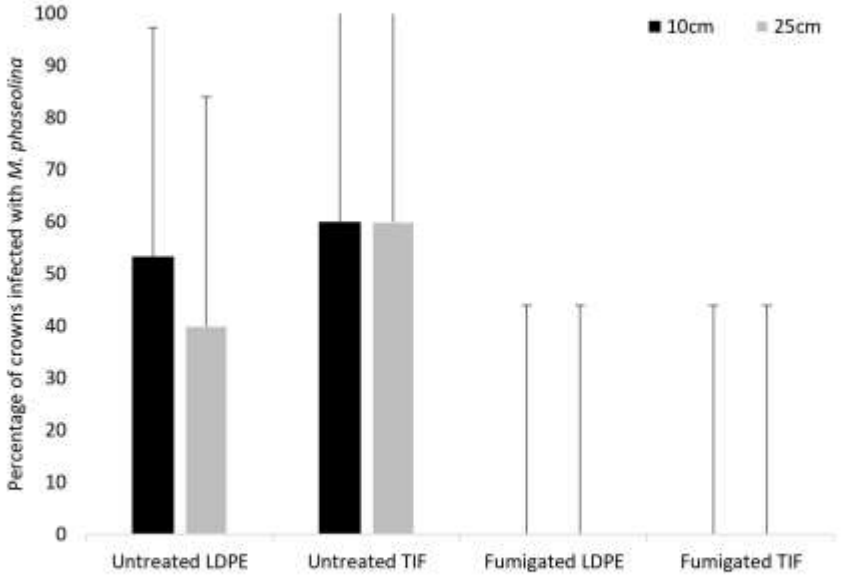

Figure 3. Percentage of strawberry crowns infected with *M. phaseolina*, post fumigation. Bars represent LSDs where p = 0.05.

4.1.2.3. *M. phaseolina* concentrations in soil

Fumigation significantly reduced the concentration of *M. phaseolina* in the soil by an average of 44%. (Figure 4). There was no significant difference in *M. phaseolina* concentrations in soil between the sampling depths (p = 0.983) or plastic film treatments (p = 0.692).

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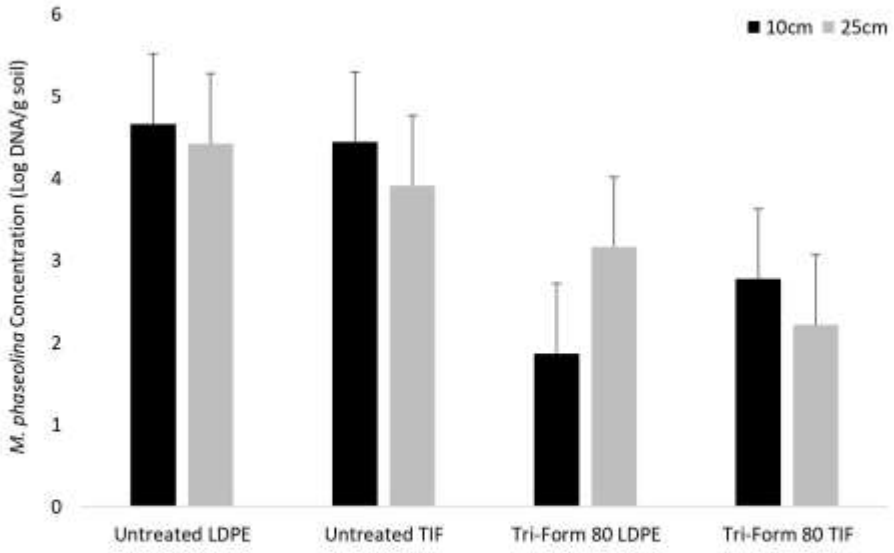

Figure 4. The average concentrations of *M. phaseolina* log DNA copies/g of soil. Bars represent LSDs where p = 0.05.

4.1.3. Conclusions

(a) When fumigating with Tri-Form® 80, soil sealing with TIF retained higher concentrations of 1,3 dichloropropene in soil than LDPE. Higher concentrations of fumigants in soil, over longer periods of time, have the potential to increase control of soil-borne pathogens.

(b) In this trial, fumigation under TIF did not increase control *M. phaseolina*, in soil or buried crowns, compared with fumigation under LDPE. This is probably because the structure of the columns helped to retain relatively high concentrations of fumigants in all Tri-Form® 80 treatments.

(c) This experiment showed a potential for TIF to improve fumigant concentrations in soils in the field and consequently the control of charcoal rot. This experiment was repeated (see section 4.6.).

4.2. Soil column experiment 2: Effectiveness of metham sodium and Tri-Form® 80 against *M. phaseolina* in soil

Completed: 5/2/2018.

4.2.1. Aims

To determine if co-application of metham sodium and Tri-Form® 80 (TF80) would reduce the amount of *M. phaseolina* present in infected strawberry crowns that were buried in soil.

List of treatments

- Tri-Form® 80 (400 kg/ha) under TIF.
- Co-application of Tri-Form® 80 (400 kg/ha) with Metham Sodium (200L/ha) under TIF. Note: these products must be co-applied because they can be explosive in cylinders when mixed.
- Metham Sodium (200L/ha) under TIF.
- Untreated under TIF.

4.2.2. Results

4.2.2.1. Fumigant concentrations

In columns treated with Tri-Form® 80 and/or metham, there were no significant differences between the treatments or soil depths in the concentrations of 1,3-dichloropropene, chloropicrin and/or metham sodium (Figures 5, 6 and 7).

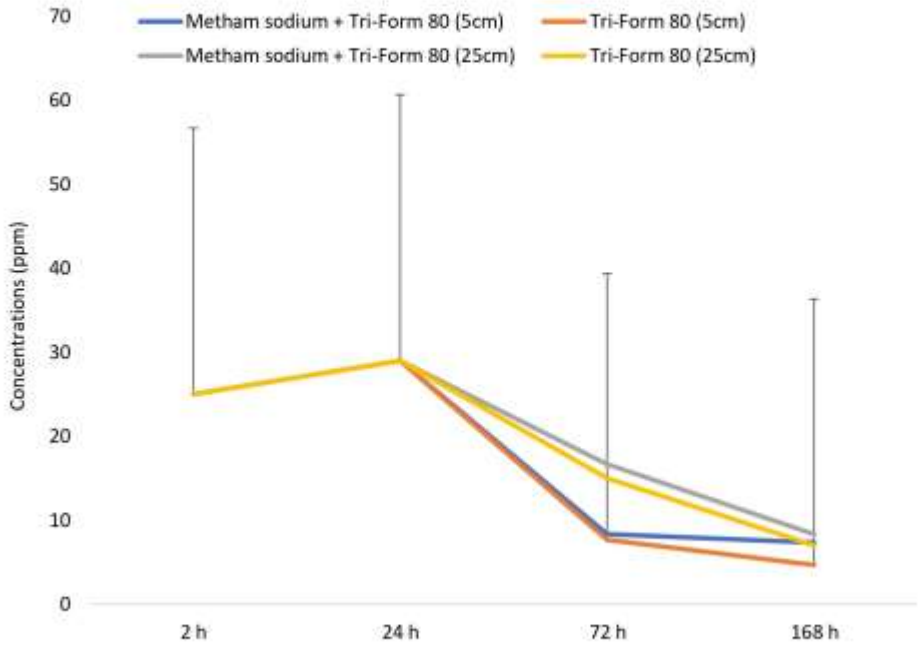

Figure 5. Concentrations of 1,3-dichloroproene inside soil columns treated with Tri-Form® 80, post fumigation. Bars represent LSDs where p = 0.05.

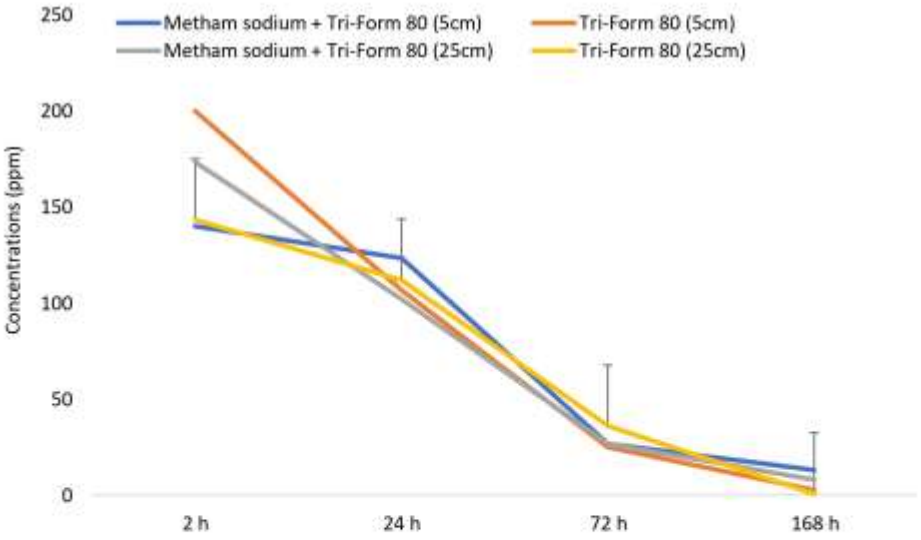

Figure 6. Concentrations of chloropicrin inside soil columns treated with Tri-Form® 80, post fumigation. Bars represent LSDs where p = 0.05.

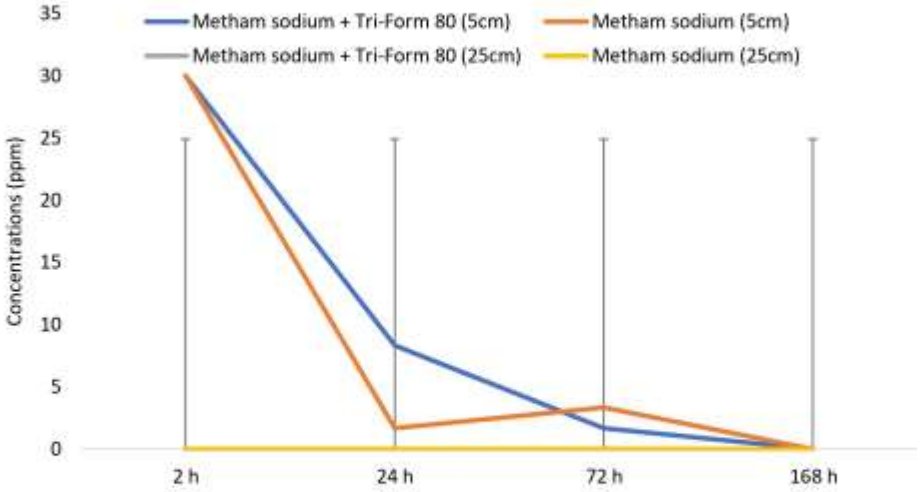

Figure 7. Concentrations of methyl isothiocyanate (active degradation product of metham sodium) inside soil columns treated with metham sodium, post fumigation. Bars represent LSDs where p = 0.05.

4.2.2.2. Pathogen viability in crowns

There were high levels of variation in the viability of *M. phaseolina* isolated from buried crowns in this experiment. Consequently, there were no significant differences in the viability of the pathogen in crowns between treatments (p = 0.148) or sampling depths (Figure 8).

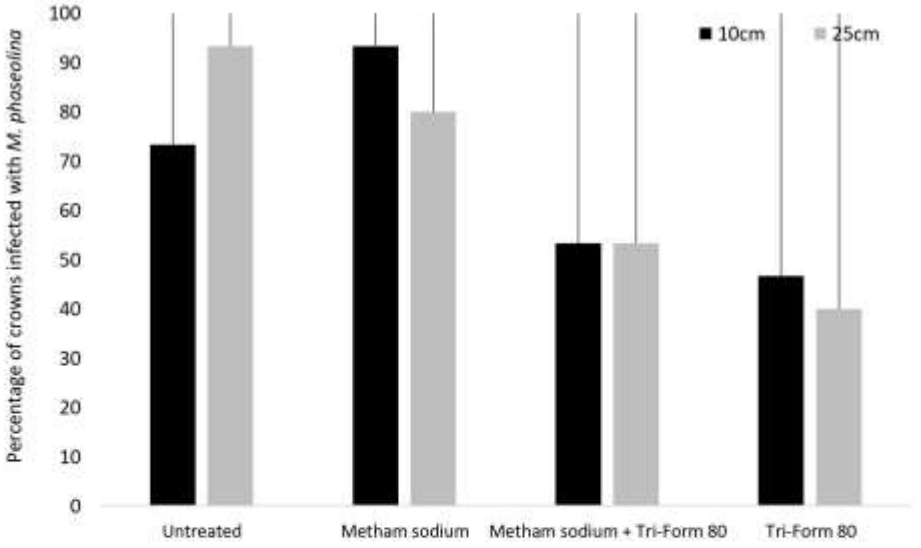

Figure 8. Percentage of strawberry crowns infected with *M. phaseolina*, post fumigation. Bars represent LSDs where p = 0.05.

4.2.2.3. *M. phaseolina* concentrations in soil

The TF80 + metham sodium treatment significantly reduced the amount of *M. phaseolina* DNA present in soils compared with all other treatments (Figure 9). There was no significant difference in concentrations of *M. phaseolina* in soil between sampling depths (p = 0.678).

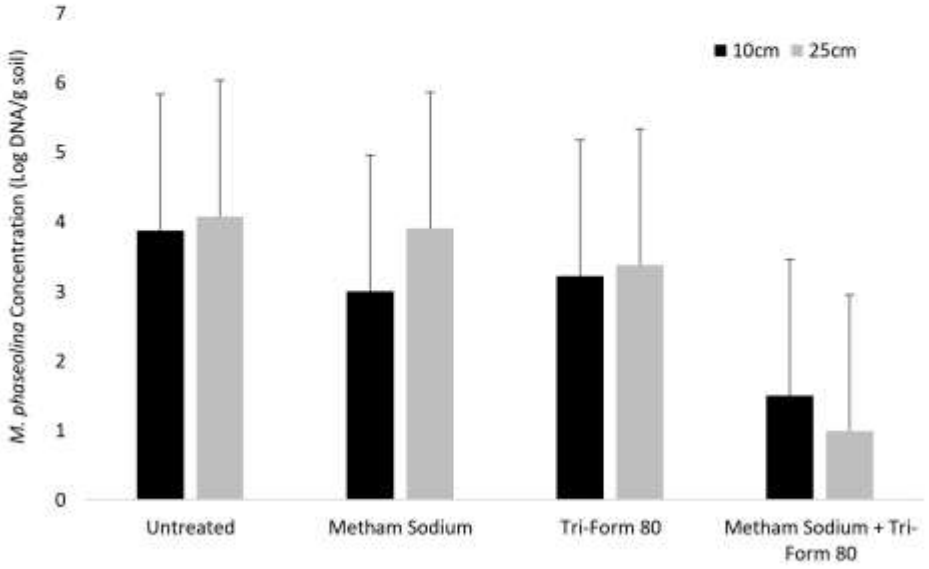

Figure 9. The average concentrations of *M. phaseolina* log DNA copies/g of soil. Bars represent LSDs where p = 0.05.

4.2.3. Conclusions

(a) TF80 + metham sodium reduced the amount of *M. phaseolina* in soils compared with either fumigant applied alone.

(b) Results from this experiment indicate that co-application of metham sodium and Tri-Form® 80 may have a synergistic effect in controlling *M. phaseolina* and therefore this combination was investigated in a field trial (see section 5.4.).

4.3. Soil column experiment 3: Effectiveness of dimethyl disulphide and chloropicrin against *M. phaseolina* in soil

Completed: 27/3/2018.

4.3.1. Aims

To determine the effectiveness of dimethyl disulphide (DMDS) and chloropicrin (PIC) against *M. phaseolina*, when applied either alone or together. Dimethyl disulphide is non-registered soil fumigant in Australia that has been shown to control charcoal rot in California, USA.

List of treatments

- Dimethyl disulphide (480 L/ha) under TIF.
- Chloropicrin (200 L/ha) under TIF.
- Dimethyl disulphide (480 L/ha) + Chloropicrin (200 L/ha) under TIF.
- Untreated under TIF.

4.3.2. Results

4.3.2.1. Fumigant concentrations

Soil depth had a significant effect on the concentrations of dimethyl disulphide, which were higher at 5cm than at 25cm ($p > 0.05$). The concentrations of dimethyl disulphide in soil were significantly higher in the dimethyl disulphide + chloropicrin treatment compared with dimethyl disulphide treatment by 18% (Figure 10). The concentrations of chloropicrin were significantly higher in the chloropicrin treatment than the dimethyl disulphide + chloropicrin treatment by 23% (Figures 11).

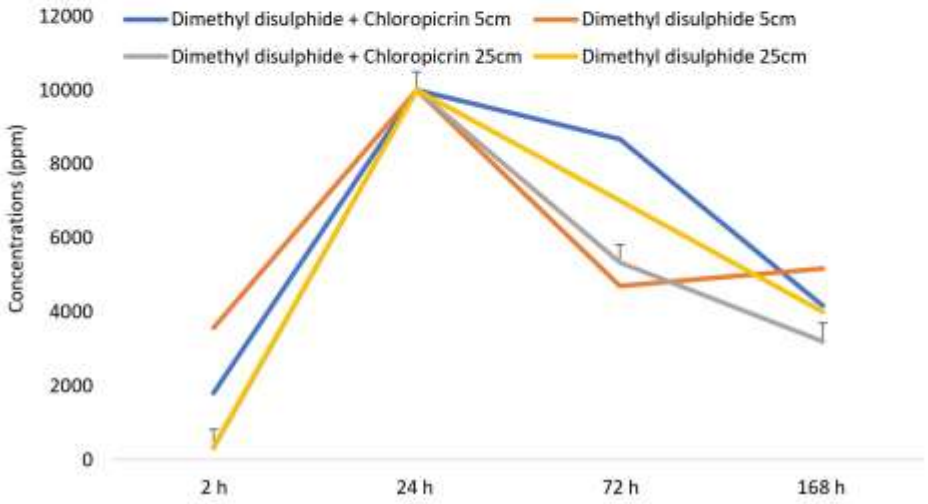

Figure 10. Concentrations of dimethyl disulphide inside soil columns, post fumigation. Bars represent LSDs where p = 0.05.

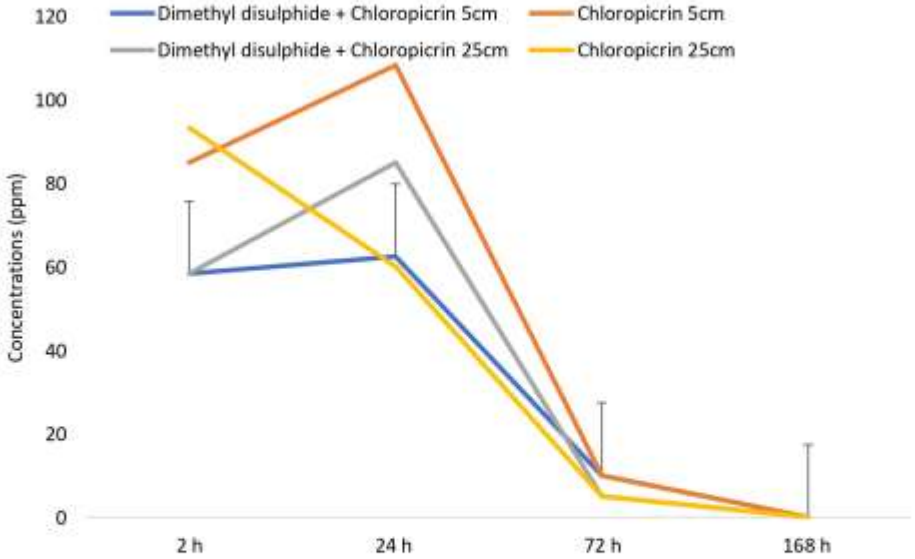

Figure 11. Concentrations of chloropicrin inside soil columns, post fumigation. Bars represent LSDs where p = 0.05.

4.3.2.2. Pathogen viability in crowns

The chloropicrin and dimethyl disulphide + chloropicrin treatments significantly reduced the viability of *M. phaseolina* inside strawberry crowns compared with dimethyl disulphide alone and the untreated control, irrespective of soil depth (Figure 12).

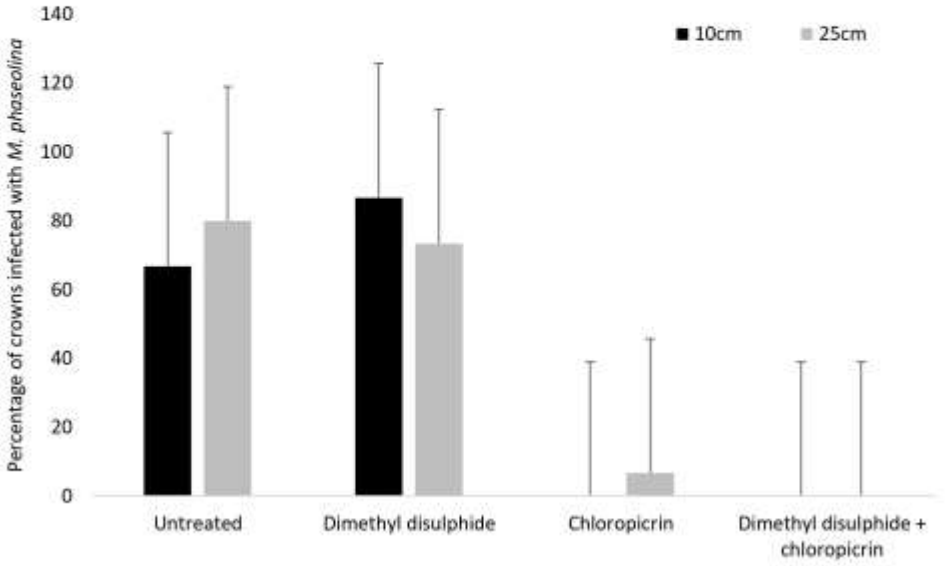

Figure 12. Percentage of strawberry crowns infected with *M. phaseolina*, post fumigation. Bars represent LSDs where p = 0.05.

4.3.2.3. *M. phaseolina* concentrations in soil

The dimethyl disulphide + chloropicrin treatment reduced the concentrations of *M. phaseolina* DNA present in soils compared with either fumigant applied alone and the untreated control (Figure 13).

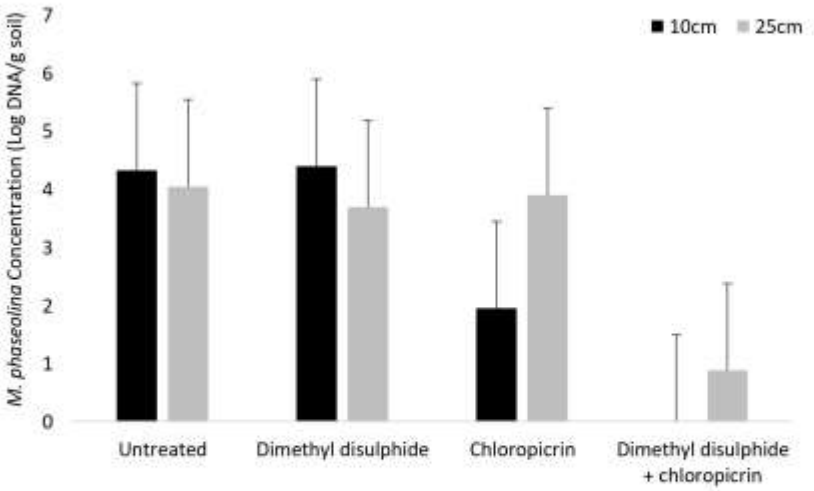

Figure 13. The average concentrations of *M. phaseolina* log DNA copies/g of soil. Bars represent LSDs where p = 0.05.

4.3.3. Conclusions

(a) Applying both dimethyl disulphide and chloropicrin was more efficacious against *M. phaseolina* in soil, compared with either product applied alone.

(b) Mixtures of dimethyl disulphide and chloropicrin have strong potential for controlling charcoal rot in the field. The product has a strong odour, and this requires careful consideration before conducting large-scale trials in the field (e.g. use of photocatalytic films, odour masking agents). Field trials were not conducted in this project because there was no decision from the chemical company to register the product in Australia.

4.4. Soil column experiment 4: Effectiveness of fumigation against *M. phaseolina* within whole and split strawberry crowns

Completed: 22/8/2018.

4.4.1. Aims

To determine the impact of cutting strawberry crowns (to simulate mulching crowns in the field) on control of *M. phaseolina* by a standard soil fumigant (Tri-Form® 80).

List of treatments

- Tri-Form® 80 (400 kg/ha) under LDPE (Half crowns).
- Tri-Form® 80 (400 kg/ha) under LDPE (Whole crowns).
- Untreated under LDPE (Half crowns).
- Untreated under LDPE (Whole crowns).

4.4.2. Results

4.4.2.1. Fumigant concentrations

Concentrations of 1,3-dichloropropene were significantly higher at depths of 5cm compared with 25cm ($p < 0.001$), by an average of 47% (Figure 15). Depth had no effect on the concentrations of chloropicrin, throughout the study period (Figure 14).

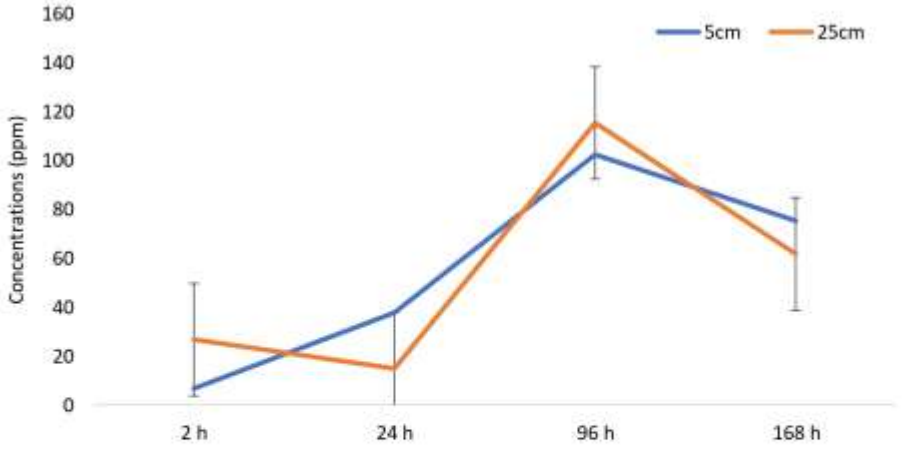

Figure 14. Concentrations of chloropicrin inside soil columns treated with Tri-Form® 80, post fumigation. Bars represent LSDs where p = 0.05.

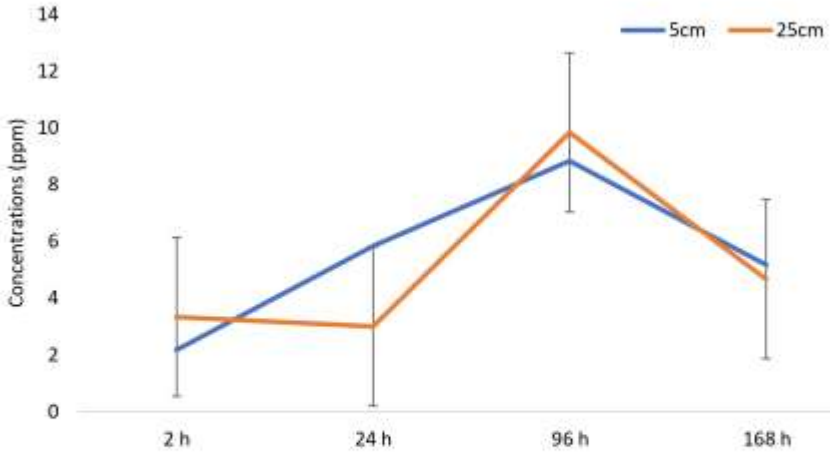

Figure 15. Concentrations of 1,3-dichloropropene inside soil columns treated with Tri-Form® 80, post fumigation. Bars represent LSDs where p = 0.05.

4.4.2.2. Pathogen viability in crowns

The fumigated treatments reduced the viability of *M. phaseolina* inside strawberry crowns compared with the untreated controls (p = 0.009). There was no significant difference in the viability of *M. phaseolina* in crowns between sampling depths (p = 0.552). Tri-Form® 80 significantly reduced the viability of *M. phaseolina* in half crowns by 53% (Figure 16).

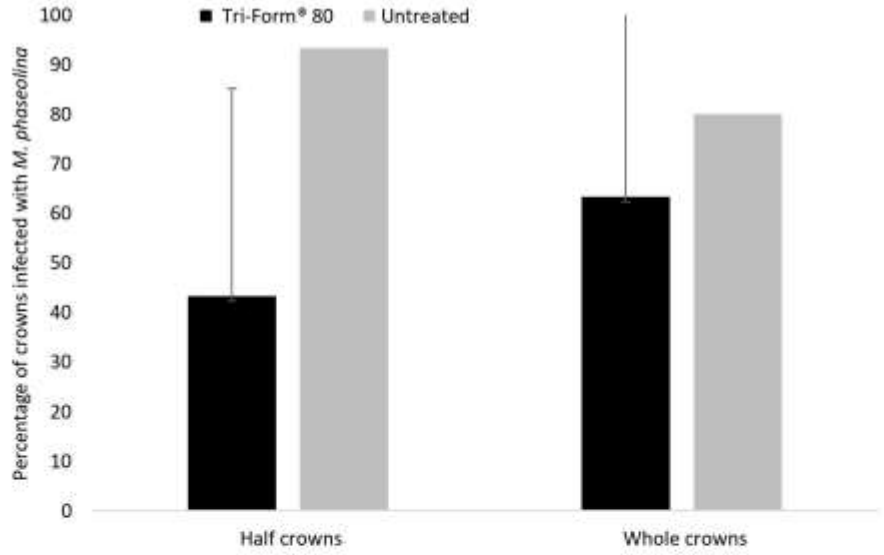

Figure 16. Percentage of strawberry crowns infected with *M. phaseolina*, post fumigation. Bars represent LSDs where p = 0.05.

4.4.2.3. *M. phaseolina* concentrations in soil

Fumigation significantly reduced the amount of *M. phaseolina* DNA in soils compared with the untreated controls by 39%, for treatments containing either half or whole crowns (Figure 17). There was no significant difference in the concentrations of *M. phaseolina* in soil between sampling depths (p = 0.311).

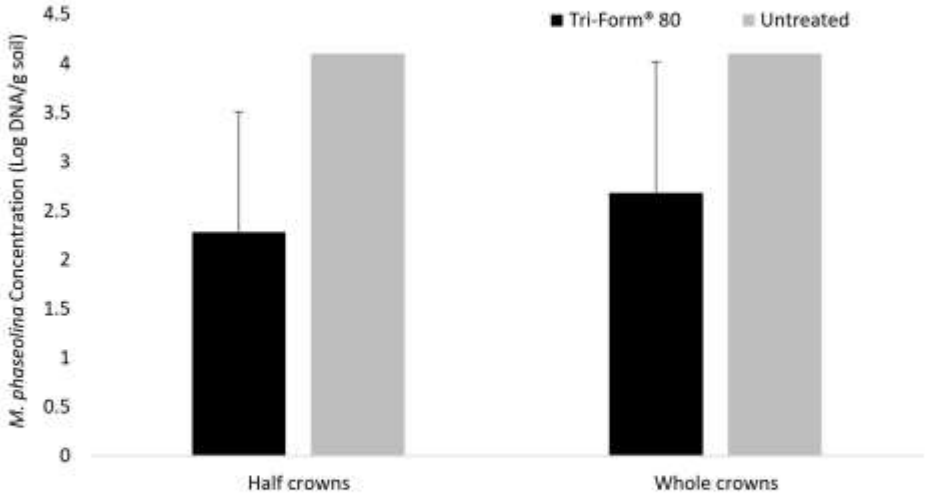

Figure 17. The average concentrations of *M. phaseolina* log DNA copies/g of soil. Bars represent LSDs where p = 0.05.

4.4.3. Conclusions

(a) Tri-Form® 80 control *M. phaseolina* more effectively in cut crowns, that were buried in soil, than in whole crowns.

(b) These results suggest that the mechanical destruction of crowns in the field (e.g. rotary hoeing) may increase the efficacy of standard fumigant treatments.

4.5. Soil column experiment 5: Effectiveness of fumigation with methyl iodide and chloropicrin against *M. phaseolina*

Completed: 24/5/2019.

4.5.1. Background

Methyl iodide is a soil fumigant that is not currently registered in Australia. Previous research shows that it is highly effective at controlling *Macrophomina* in soil. However, further research would be required to support its potential registration in Australia, particularly data on its effectiveness in mixture with chloropicrin.

4.5.2. Aims

To determine the effectiveness of methyl iodide and chloropicrin for control of *M. phaseolina* in soil, when applied alone or together at different concentrations.

List of treatments

- MI/PIC 70/30 - Methyl iodide 70% + Chloropicrin 30% (500 kg/ha) under LDPE.
- MI.PIC 50/50 - Methyl iodide 50% + Chloropicrin 50% (500 kg/ha) under LDPE.
- MI - Methyl iodide (500 kg/ha) under LDPE.
- PIC - Chloropicrin (500kg/ha) under LDPE.
- Untreated under LDPE.

4.5.3. Results

4.5.3.1. Fumigant concentrations

Depth did not impact the concentrations of methyl iodide or chloropicrin, across the study period (Figures 18 and 19).

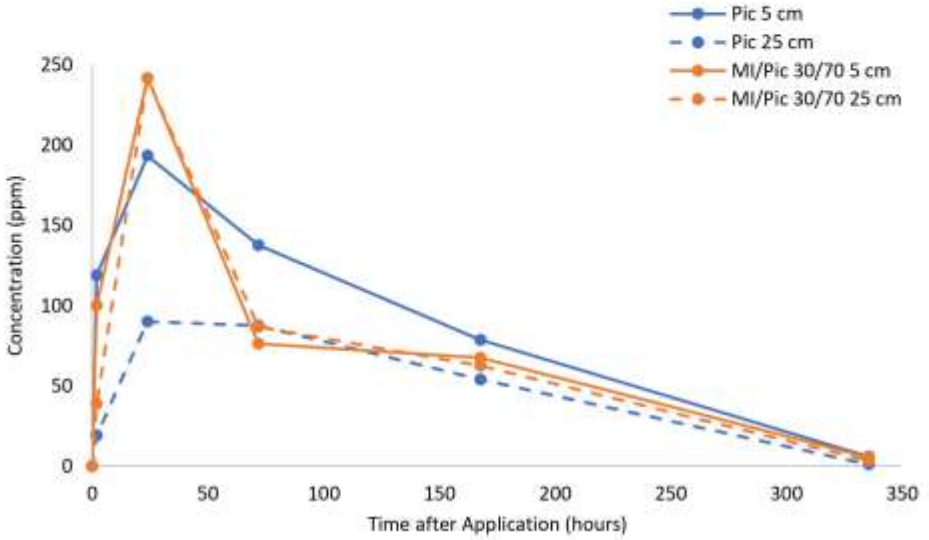

Figure 18. Concentrations of chloropicrin inside soil columns, post fumigation.

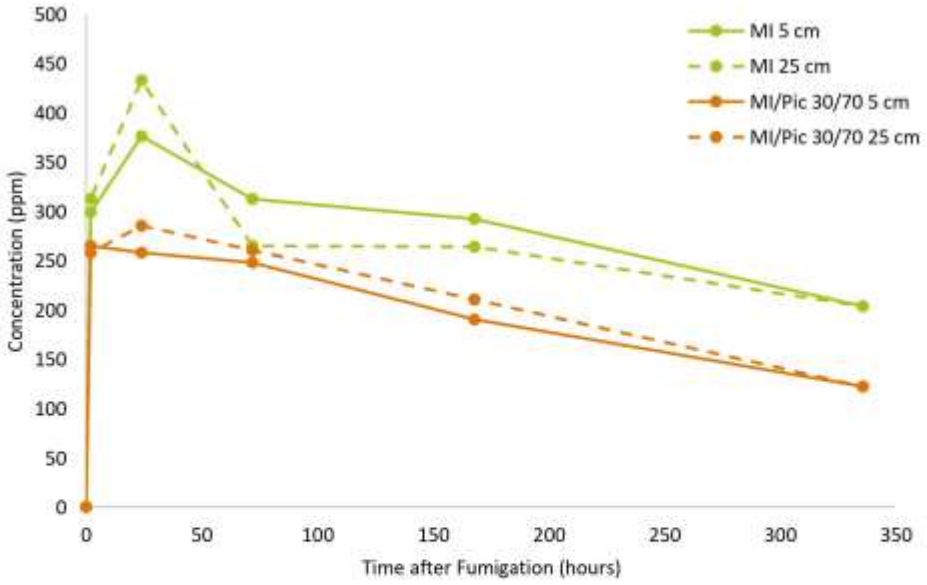

Figure 19. Concentrations of methyl iodide inside soil columns, post fumigation.

4.5.3.2. Pathogen viability in crowns

The fumigated treatments significantly reduced the viability of *M. phaseolina* inside strawberry crowns compared with the untreated controls ($p < 0.05$), at both sampling depths (Figure 20).

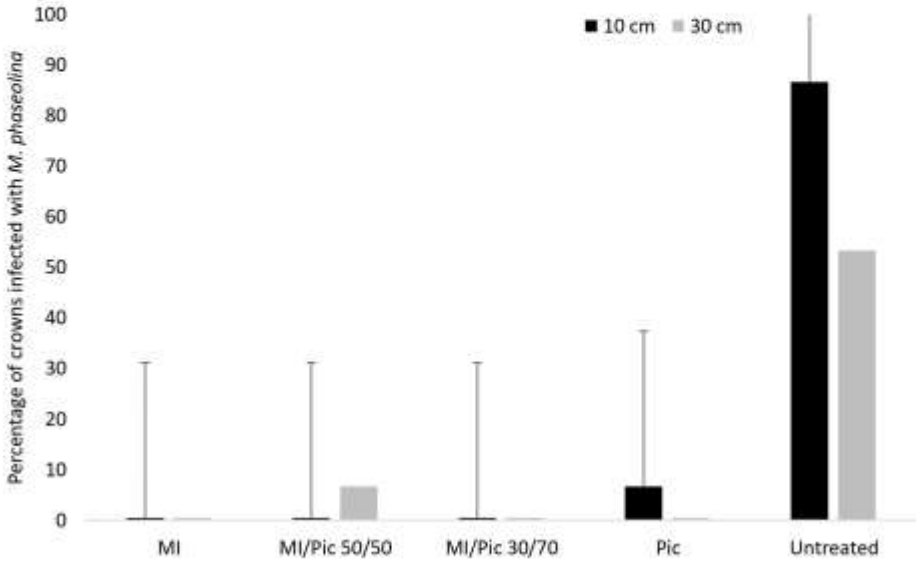

Figure 20. Percentage of strawberry crowns infected with *M. phaseolina*, post fumigation. Bars represent LSDs where $p = 0.05$.

4.5.3.3. *M. phaseolina* concentrations in soil

The treatments containing methyl iodide significantly reduced the amount of *M. phaseolina* DNA in soils compared with chloropicrin alone and untreated (Figure 21).

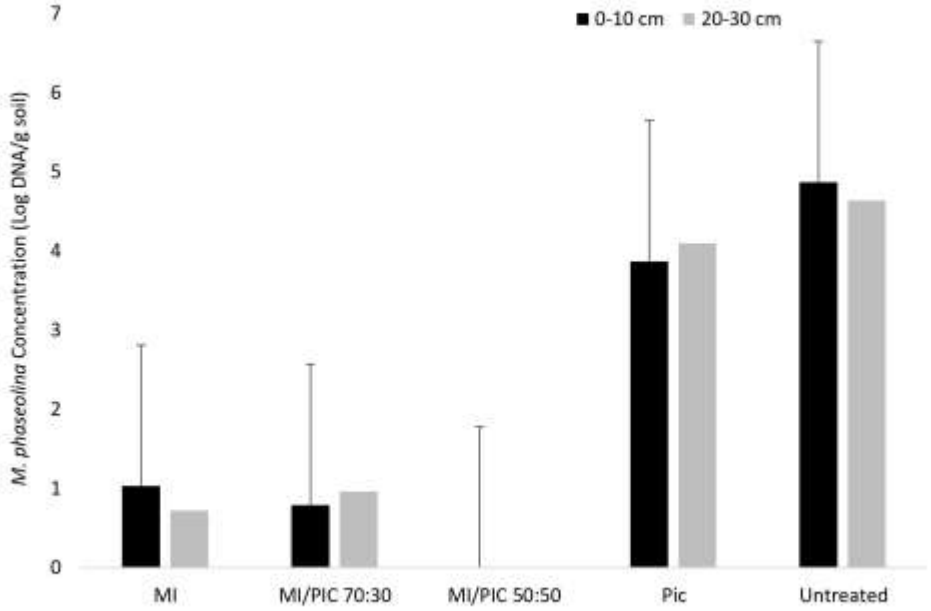

Figure 21. The average concentrations of *M. phaseolina* log DNA copies/g of soil. Bars represent LSDs where p = 0.05.

4.5.4. Conclusions

(a) Treatments containing methyl iodide significantly reduced the amount of *M. phaseolina* in the soil, compared with chloropicrin alone and the untreated control.

(b) Methyl iodide was not available for field trials during this project, but shows strong promise as a potential control for charcoal rot and as an alternative to methyl bromide.

4.6. Soil column experiment 6: Effectiveness of totally impermeable films (TIFs) for retaining fumigants in soil

Completed: 1/11/2019.

4.6.1. Aims

To determine if totally impermeable film (TIF) would retain higher concentrations of the fumigant Tri-Form® 80 in soil, compared with low density polyethylene (LDPE) film.

List of treatments

- Tri-Form® 80 (400 kg/ha) under TIF.
- Tri-Form® 80 (400 kg/ha) under LDPE.
- Untreated under LDPE.

4.6.2. Amended methods

- Fumigant concentrations were recorded, at 2, 24, 72, 168 and 336hrs after fumigation, from depths of 5cm and 25cm (Gastec® indicator tubes).

4.6.3. Results

4.6.3.1. Fumigant concentrations

TIF retained significantly more chloropicrin ($p < 0.001$) and 1,3-dichloropropene ($p = 0.03$) in soil than LDPE by 32% and 11%, respectively (Figures 22 and 23). There was no significant difference in the concentrations of chloropicrin ($p = 0.709$) or 1,3-dichloropropene ($p = 0.305$) between the two sampling depths.

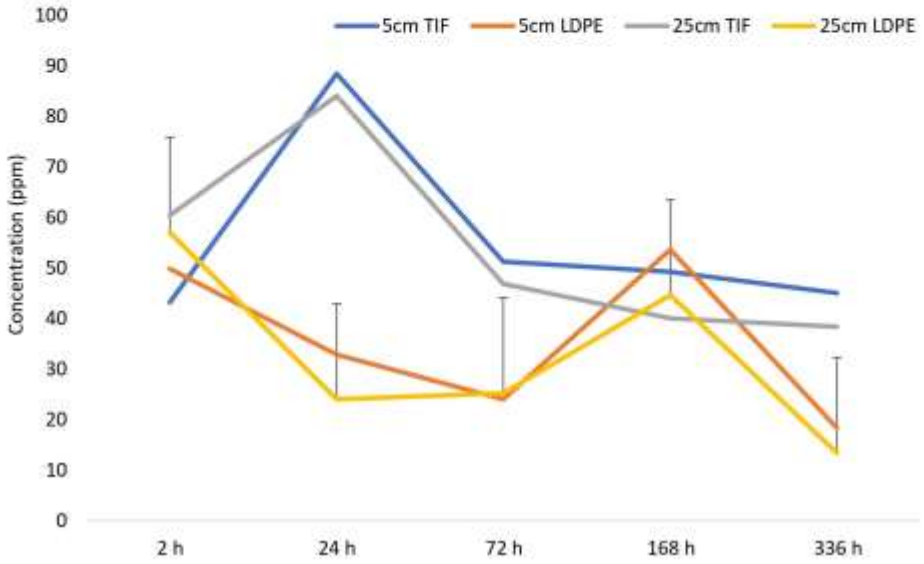

Figure 22. Concentrations of chloropicrin inside soil columns treated with Tri-Form® 80, post fumigation. Bars represent LSDs where $p = 0.05$.

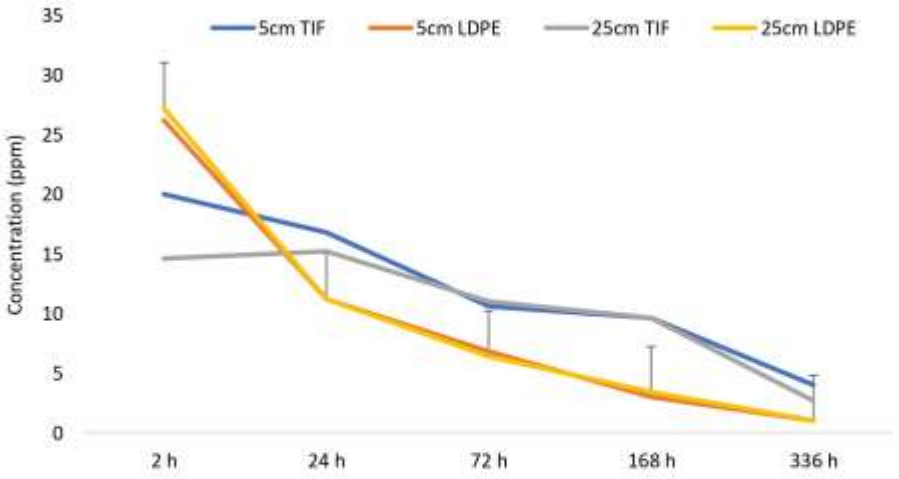

Figure 23. Concentrations of 1,3-dichloropropene inside soil columns treated with Tri-Form® 80, post fumigation. Bars represent LSDs where $p = 0.05$.

4.6.3.2. Pathogen viability in crowns

Fumigation with Tri-Form® 80 significantly reduced the viability of *M. phaseolina* in buried crowns by 90% compared with the untreated control ($p < 0.001$) (Figure 24). The method of sealing soils did not significantly affect the viability of *M. phaseolina* in buried crowns.

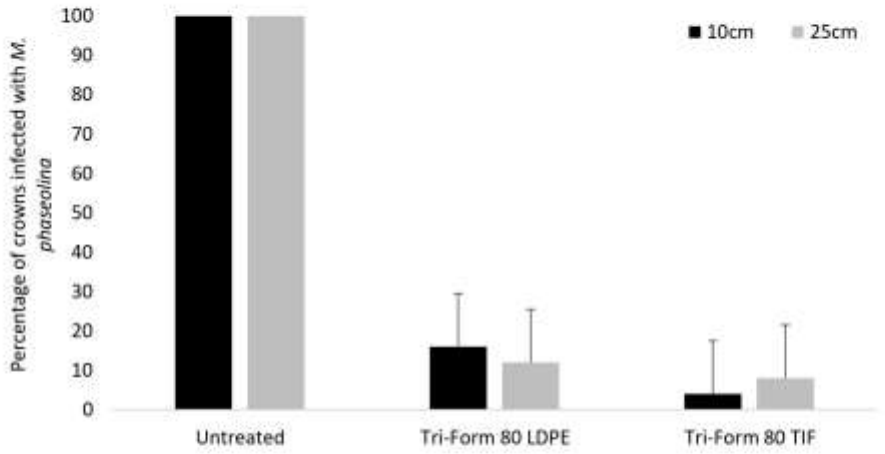

Figure 24. Percentage of strawberry crowns infected with *M. phaseolina*, post fumigation. Bars represent LSDs where $p = 0.05$.

4.6.3.3. *M. phaseolina* concentrations in soil

Fumigation with Tri-Form® 80 significantly reduced the concentration of *M. phaseolina* in soil by at least 86% compared with the untreated control ($p < 0.001$) (Figure 25). The method of sealing soils did not significantly affect the concentration of *M. phaseolina* in the soil.

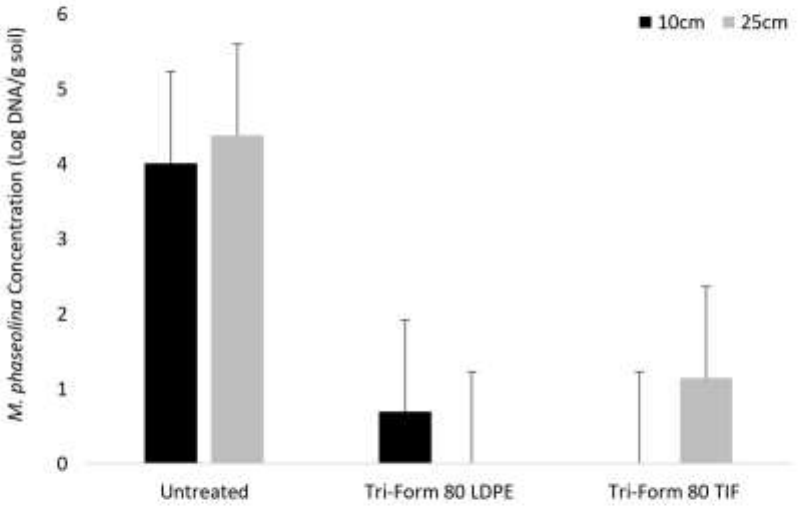

Figure 25. The average concentrations of *M. phaseolina* log DNA copies/g of soil. Bars represent LSDs where $p = 0.05$.

4.6.4. Conclusions

(a) Results from this experiment confirm earlier results (see section 4.1) that TIF retains fumigants at higher concentrations and for greater periods, compared with LDPE.

(b) Despite this result, TIF was no more effective at controlling *M. phaseolina*, in soil or buried crowns, than LDPE in this experiment. This is probably because the structure of the columns helped to retain relatively high concentrations of fumigants in all Tri-Form® 80 treatments. Results supported further evaluation of the use of TIFs with fumigants under field conditions (see section 5.1 and 5.2).

5. Field experiments

5.1. Field experiment 1: Effectiveness of different applications techniques for EDN for control of charcoal rot

Location: Coldstream, Victoria

Fumigated: 21/12/2017

Planted: 26/2/2018

First Fruiting Period: 23/4/2018 -21/06/2019

Second Fruiting Period: 23/08/2018 – 23/04/2019

5.1.1. Aims

To determine if ethanedinitrile (EDN) controls charcoal rot of strawberry more effectively when: (a) applied under TIF, compared with LDPE, (b) shank-applied compared with drip applied, and (c) co-applied with PIC. The trial also aimed to evaluate the effectiveness of MB recaptured from quarantine applications for control of charcoal rot, and the relative susceptibility of strawberry plants grown from plug or bare-rooted transplants to the disease.

5.1.2. Design

The experiment was a randomised split-plot design (Figure 26). Fumigant treatments formed the main plots (12 levels: see below) and planting material formed the split-plots (2 levels: plug or bare-rooted transplants). There were three plots of each treatment, except for EDN drip TIF and EDN + PIC TIF (blocked five times), and EDN + TF80 and TF80 (not replicated). Individual plots were 4m long with 1.5m centres.

Figure 26. Field trial 1, Coldstream, Victoria. (January 2019).

List of treatments (All treatments were planted with 10 plug and 10 bare-rooted transplants (cv. Albion), which formed the split-plot treatments)

- EDN shank TIF- Ethanedinitrile (500 kg/ha) under TIF, shank fumigation.
- EDN shank LDPE - Ethanedinitrile (500 kg/ha) under LDPE, shank fumigation. Note: this was an experimental treatment that is not permitted under the product label because EDN must be applied under TIF.
- EDN drip TIF - Ethanedinitrile (500 kg/ha) under TIF, drip fumigation.
- EDN drip LDPE - Ethanedinitrile (500 kg/ha) under LDPE, drip fumigation. Note: this was an experimental treatment that is not permitted under the product label because EDN must be applied under TIF.
- EDN + PIC TIF - Ethanedinitrile (500 kg/ha) under TIF, drip fumigation, co-applied with a shank fumigation of chloropicrin (300 kg/ha).
- EDN + PIC LDPE - Ethanedinitrile (500 kg/ha) under LDPE, drip fumigation, co-applied with a shank fumigation of chloropicrin (300 kg/ha). Note: this was an experimental treatment that is not permitted under the product label because EDN must be applied under TIF.
- PIC - Chloropicrin (300 kg/ha) under TIF, shank fumigation.
- PIC + MB(Q) - Chloropicrin (300 kg/ha) under TIF, shank fumigation, co-applied with methyl bromide (250 kg a.i./ha) recaptured from quarantine applications on activated charcoal, spread on the surface and incorporated to a depth of 25 cm with a rotary hoe.
- Untreated TIF - Untreated under TIF.
- Untreated LDPE - Untreated under LDPE.

5.1.3. Amended methods

- No fumigant concentrations were recorded during this trial.

5.1.4. Results

5.1.4.1. Pathogen viability in crowns

No fumigant treatment provided significant control of *M. phaseolina* in buried crowns compared with the control (Figure 27).

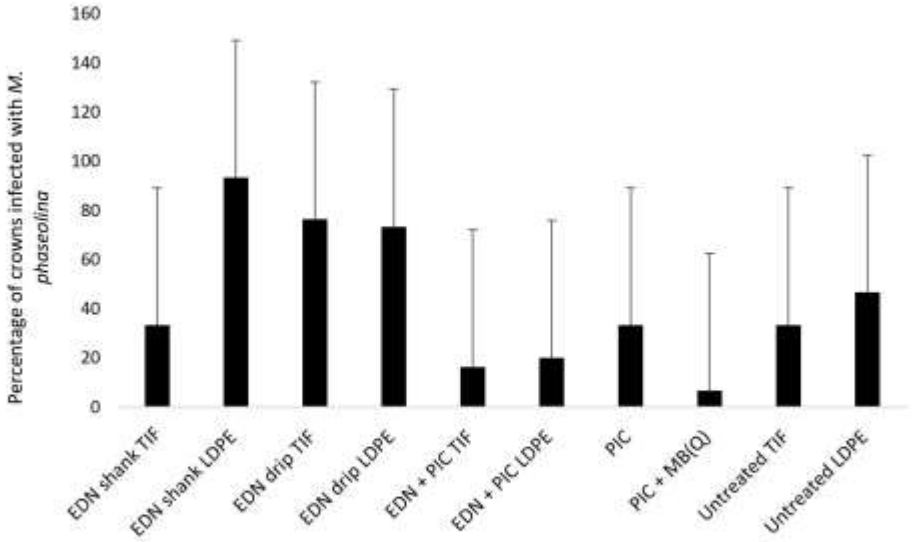

Figure 27. Percentage of strawberry crowns infected with *M. phaseolina*, post fumigation, in a field trial at Coldstream, Victoria. Bars represent LSDs where p = 0.05.

5.1.4.2. *M. phaseolina* concentrations in soil

Following fumigation (26/2/2018), all treatments except PIC, significantly reduced (to undetectable levels) concentrations of *M. phaseolina* DNA in the soil compared with the untreated controls. By the end of the experiment (>12 months), *M. phaseolina* had recolonised soils in the EDN drip and MB(Q) + PIC treatments (Table 3).

Table 3. The average concentrations of *M. phaseolina* in soil (log DNA copies/g of soil) in a field trial at Coldstream, Victoria. Soil samples were collected at depths of 0-10cm from February 2018-May 2019. The ordinal symbols represent significant differences, where p = 0.05, in each column. The least significant difference (LSD) values are provided.

Treatment	Date							
	26/02/18	12/04/18	29/08/18	28/11/18	12/12/18	17/01/19	7/03/19	17/05/19
PIC	1.22 ^{ab}	3.39 ^{b}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}
PIC + MB(Q)	0 ^{a}	0.8b ^{ab}	0 ^{a}	2.64 ^{b}	0 ^{a}	0.89 ^{ab}	0.90 ^{ab}	1.54 ^{ab}
Untreated TIF	2.69 ^{b}	1.96 ^{b}	0 ^{a}	0.97 ^{ab}	0 ^{a}	2.17 ^{b}	1.03 ^{ab}	2.11 ^{ab}
Untreated LDPE	1.89 ^{b}	1.76 ^{ab}	1.25 ^{b}	0.90 ^{ab}	1.01 ^{a}	2.05 ^{ab}	2.88 ^{b}	1.06 ^{ab}
EDN + PIC TIF	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0.46 ^{ab}	0 ^{a}	0 ^{a}
EDN shank LDPE	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	1.37 ^{ab}	0 ^{a}	0 ^{a}
EDN + PIC LDPE	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}
EDN drip TIF	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0.95 ^{ab}	1.59 ^{ab}
EDN shank TIF	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0.87 ^{a}	0 ^{a}	1.16 ^{ab}	0 ^{a}
EDN drip LDPE	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	2.27 ^{ab}	3.00 ^{b}
LSD	1.45	1.32	1.1	1.1	ns	2.1	2.54	2.48

5.1.4.3. Charcoal rot incidence and severity

Significant levels of charcoal rot first developed in plants during February. At the end of the experiment all fumigant treatments significantly reduced disease compared with the untreated controls, except for EDN drip TIF, EDN drip LDPE and PIC + MB(Q). Results at the end of the trial showed that there was no significant difference in disease between (a) the EDN treatments applied under TIF or LDPE, or (b) the EDN (drip) + PIC or PIC treatments (Figure 28). There was no significant difference in disease in strawberry plants derived from plugs or bare-rooted transplants (p = 0.672 at the end of the study).

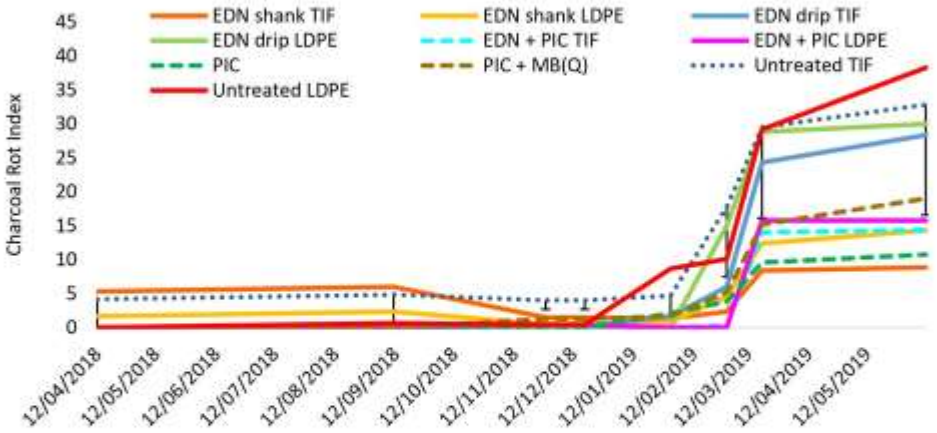

Figure 28. The index of charcoal rot in strawberry grown in different treatments in a field trial at Coldstream, Victoria. Disease assessments were conducted at regular intervals from April 2018-June 2019. The bars represent the least significant difference, where p = 0.05.

5.1.4.4. Weed assessments

There was no significant difference in weed emergence between the EDN treatments applied (a) under TIF or LDPE, (b) by drip or shank, or (c) when co-applied with PIC (Figure 29).

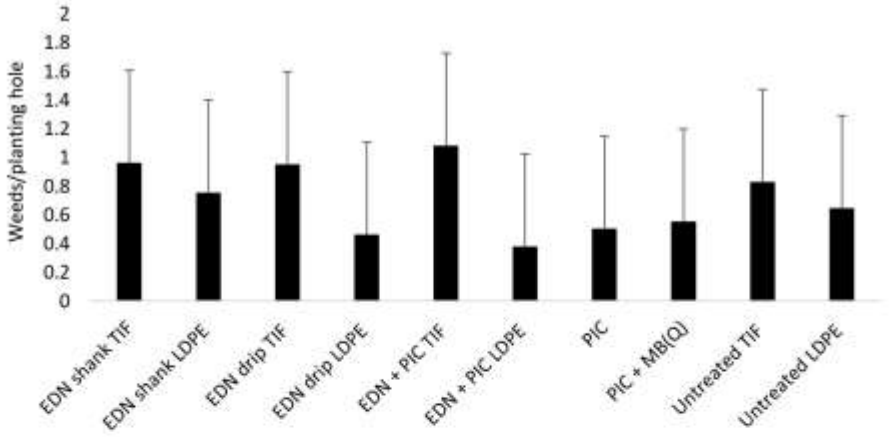

Figure 29. The number of weeds that emerged per strawberry planting hole, in a field trial at Coldstream, Victoria. Bars represent LSDs where p = 0.05.5.1.4.5. Fruit yields and revenue

EDN + PIC TIF and EDN shank LDPE significantly increased marketable fruit yield by 88% and 80%, respectively, compared with the TIF control (Table 4). EDN+PIC TIF and EDN shank LDPE significantly increased revenue from fruit by 87% compared with the TIF control.

There was no significant difference between the plugs and bare-rooted transplants, regarding their cumulative total fruit yield (average of 515.3g/plant and 535g/plant, respectively), marketable fruit yield (average of 257.4g/plant and 285.2g/plant, respectively), total number of fruit (average of 38/plant for both), marketable number of fruit (average of 16/plant and 17/plant, respectively), average fruit size (average of 16.29g and 17.02g, respectively) and revenue from fruit (average of $1.5/plant and $1.64/plant, respectively). However,

plugs significantly reduced profits compared with the bare-rooted transplants, due to their increased cost ($0.60 more costly).

Table 4. Cumulative strawberry fruit production per plant in different fumigant treatments (23/04/2018-23/4/2019) in a trial at Coldstream, Victoria. The ordinal symbols represent significant differences, where p = 0.05, in each column. The costs associated with the treatments (e.g. fumigation) have not been deducted from the marketable fruit revenue data. The least significant difference (LSD) values are provided.

Treatment	Total fruit yield (g)	Marketable fruit yield (g)	Total number of fruit	Marketable number of fruit	Marketable fruit size (g/berry)	Marketable fruit revenue (AU$)
EDN shank TIF	599.3^{a}	288^{ab}	40.28^{ab}	16.39^{ab}	17.59^{ab}	1.75^{ab}
EDN shank LDPE	618.4^{a}	323.8^{b}	42.45^{ab}	18.21^{ab}	17.52^{ab}	1.94^{b}
EDN drip TIF	496.1^{a}	266.3^{ab}	38.25^{ab}	16.38^{ab}	16.19^{ab}	1.5^{ab}
EDN drip LDPE	434.7^{a}	242.4^{ab}	36.3^{ab}	15.71^{ab}	15.39^{ab}	1.36^{ab}
EDN + PIC TIF	623.8^{a}	337.7^{b}	44.75^{b}	19.4^{b}	17.31^{ab}	1.93^{b}
EDN + PIC LDPE	534.9^{a}	285.1^{ab}	40.6^{ab}	16.79^{ab}	16.99^{ab}	1.63^{ab}
PIC	579.7^{a}	289.8^{ab}	38.05^{ab}	15.96^{ab}	18.13^{b}	1.72^{ab}
PIC + MB(Q)	536.9^{a}	260.5^{ab}	36.47^{ab}	15.83^{ab}	16.17^{ab}	1.54^{ab}
Untreated TIF	389.7^{a}	178.9^{a}	28.23^{a}	11.72^{a}	15.27^{a}	1.03^{a}
Untreated LDPE	408.3^{a}	210.1^{a}	32.22^{ab}	13.09^{ab}	15.86^{ab}	1.23^{ab}
LSD	ns	140.2	14.3	7.03	2.78	0.85

5.1.5. Conclusions

(a) Most fumigants reduced concentrations of *M. phaseolina* in soil following treatment.

(b) Some treatments containing EDN were effective in increasing fruit yields and revenue from fruit compared with the untreated controls.

(c) There was no difference in charcoal rot or yields in strawberry plants grown under TIF or LDPE.

(d) There was no difference in charcoal rot or yields in strawberry plants grown in soils that were either shank or drip fumigated.

(e) There was no difference in charcoal rot or yields in strawberry plants grown from plug or bare-rooted transplants.

(f) MB(Q) did not reduce disease or increased fruit yields compared with the untreated controls.

(g) Results from qPCR tests for *M. phaseolina* in soil did not always correlate with charcoal rot results in this trial. For example, no DNA of *M. phaseolina* was detected in plots treated with EDN + PIC LDPE, but plants in this treatment still developed charcoal rot. More research is required to understand the relationship between DNA inoculum levels of *M. phaseolina* in soil and charcoal rot in strawberry.

5.2. Field experiment 2: Effectiveness of Tri-Form® 80 (TF80) application techniques for control of charcoal rot

Location: Silvan, Victoria

Fumigated: 23/1/2018 (Broadacre treatments, see below), 1/2/2018 (Strip treatments, see below).

Planted: 22/2/2018

First Fruiting Period: 23/04/2018 – 05/06/2018

Second Fruiting Period: 12/10/2018 – 2/01/2020

5.2.1. Aims

To determine if Tri-Form® 80 (TF80) controls charcoal rot of strawberry more effectively when: (a) applied under TIF, compared with LDPE, (b) strip applied compared with broadacre applied, and (c) co-applied with DOMINUS®. DOMINUS® is a biofumigant product, meaning that the chemical compounds used in DOMINUS® were derived from plants. The trial also aimed to evaluate the relative susceptibility of strawberry plants grown from plug or bare-rooted transplants to charcoal rot.

5.2.2. Design

The experiment was a randomised split-plot design (Figure 30). Fumigant treatments formed the main plots (9 levels: see below) and planting material formed the split plots (2 levels: plug or bare-rooted transplants). There were three plots of each treatment. Individual plots were 4m long with 1.5m centres.

Figure 30. Field trial 2, Silvan, Victoria. (March 2019).

List of treatments (All treatments were planted with 10 plug and 10 bare-rooted transplants (cv. Albion), which formed the split-plot treatments)

- Broadacre LDPE - Broadacre fumigation of Tri-Form® 80 (400 kg/ha) under LDPE, shank fumigation.
- Broadacre TIF - Broadacre fumigation of Tri-Form® 80 (400 kg/ha) under TIF, shank fumigation.
- Strip TIF - Strip fumigation of Tri-Form® 80 (400 kg/ha) under TIF, shank fumigation.

- Strip LDPE - Strip fumigation of Tri-Form® 80 (400 kg/ha) under LDPE, shank fumigation.
- Strip TIF + MB(Q) - Strip fumigation of Tri-Form® 80 (250 kg/ha) under TIF, shank fumigation, co-applied with methyl bromide (250 kg a.i./ha) on activated charcoal, spread on the surface and incorporated to a depth of 25 cm with a rotary hoe.
- Strip TIF + DOMINUS - Strip fumigation of Tri-Form® 80 (400 kg/ha) under TIF, shank fumigation, co-applied with DOMINUS®.
- DOMINUS TIF - DOMINUS® (200 L/ha) under TIF, spray and incorporated to a depth of 25cm with a rotary hoe.
- Untreated TIF - Untreated under TIF.
- Untreated LDPE - Untreated under LDPE.

5.2.3. Results

5.2.3.1. Fumigant concentrations for TF80 under TIF and LDPE

The average CT value for 1,3-dichloropropene (component of TF80) under TIF (4.35ppm.h) was approximately equivalent to that under LDPE (4.42ppm.h), regardless of the application method. The average CT value for chloropicrin (component of TF80) under TIF (30.77ppm.h) was higher than that under LDPE (22.5ppm.h), regardless of the application method. However, these differences were not statistically significant.

5.2.3.2. Pathogen viability in crowns

Fumigation, except with DOMINUS®, significantly reduced the survival of *M. phaseolina* inside crowns (Figure 31).

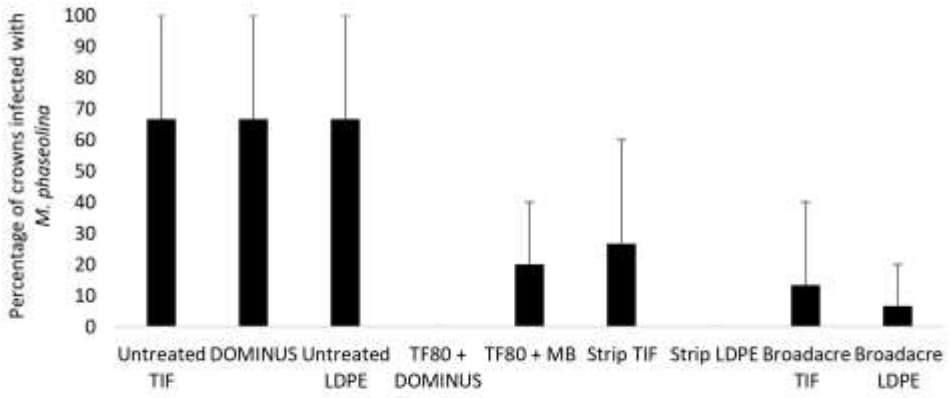

Figure 31. Percentage of strawberry crowns infected with *M. phaseolina*, post fumigation, in a field trial at Silvan, Victoria. Bars represent LSDs where p = 0.05.

5.2.3.3. *M. phaseolina* concentrations in soil

All fumigants reduced the concentration of *M. phaseolina* in the soil compared with the untreated controls (Table 5). *M. phaseolina* recolonised soils in fumigant treatments that were less effective for soil disinfestation, including TF80 + MB(Q), as early as August 2018.

Table 5. The average concentrations of *M. phaseolina* log DNA copies/g of soil in a field trial at Silvan, Victoria. Soil samples were collected at depths of 0-10cm from February 2018-December 2019. The ordinal symbols represent significant differences, where p = 0.05, in each column. The least significant difference (LSD) values are provided.

Treatment	Date								
	26/02/18	12/04/18	29/08/18	28/11/18	12/12/18	5/02/19	14/03/19	20/05/19	5/12/19
Untreated TIF	3.42^{c}	4.43^{c}	0.82^{b}	3.66^{c}	3.16^{bc}	3.6^{cd}	3.85^{c}	4.29^{b}	5.09^{b}
DOMINUS	0^{a}	0^{a}	0^{a}	1.6^{b}	0^{a}	1.74^{b}	1.12^{a}	1.4^{a}	1.25^{a}
Untreated LDPE	3.51^{c}	3.86^{c}	3.82^{d}	3.59^{c}	3.64^{c}	4.17^{d}	3.71^{c}	4.29^{b}	5.02^{b}
TF80 + DOMINUS	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	1.52^{a}
TF80 + MB(Q)	1.16^{b}	0^{a}	3.42^{d}	3.77^{c}	3.88^{c}	3.4^{cd}	3.84^{c}	4.19^{b}	4.04^{b}
Strip TIF	0.72^{ab}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.13^{a}	0^{a}	1.53^{a}
Strip LDPE	3.12^{c}	1.15^{b}	1.99^{c}	0.96^{b}	2.41^{b}	2.9^{c}	3.23^{bc}	4.27^{b}	4.26^{b}
Broadacre TIF	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0.96^{a}	2.1^{a}
Broadacre LDPE	0.82^{b}	1.0^{b}	0^{a}	0^{a}	0^{a}	0^{a}	1.78^{b}	0^{a}	1.84^{a}
LSD	1.56	1.5	1.35	1.67	1.2	0.94	1.76	1.68	2.47

5.2.3.4. Charcoal rot incidence and severity

All fumigant treatments significantly reduced charcoal rot compared with the controls, except for strip LDPE and TF80 + MB(Q) (Figure 32). The use of TIF (either strip or broadacre application) and co-application with DOMINUS® significantly and consistently reduced charcoal rot compared the industry standard of strip LDPE. There was no significant difference in disease between plug (36% disease incidence) and bare-rooted transplants (34% disease incidence), throughout the trial.

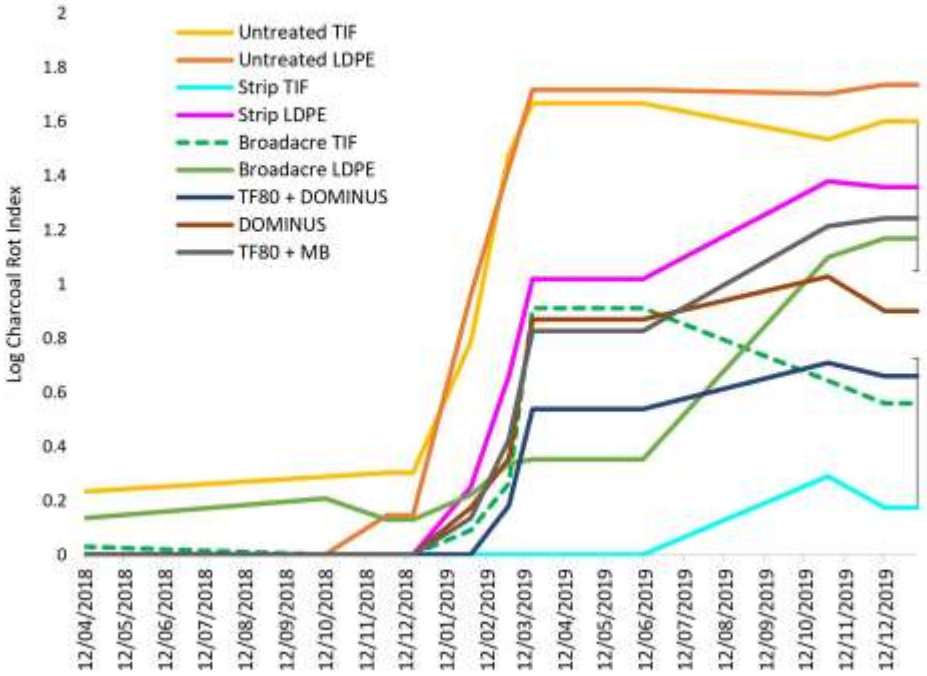

Figure 32. The index of charcoal rot in strawberry grown in different treatments in a field trial at Silvan, Victoria. Disease assessments were conducted at regular intervals from April 2018-Janruary 2020. Bars represent LSDs where p = 0.05.

5.2.3.5. Weed assessments

TF80 + DOMINUS, broadacre LDPE, broadacre TIF, TF80 + MB and strip TIF treatments significantly reduced weed emergence compared with the untreated controls (Figure 33).

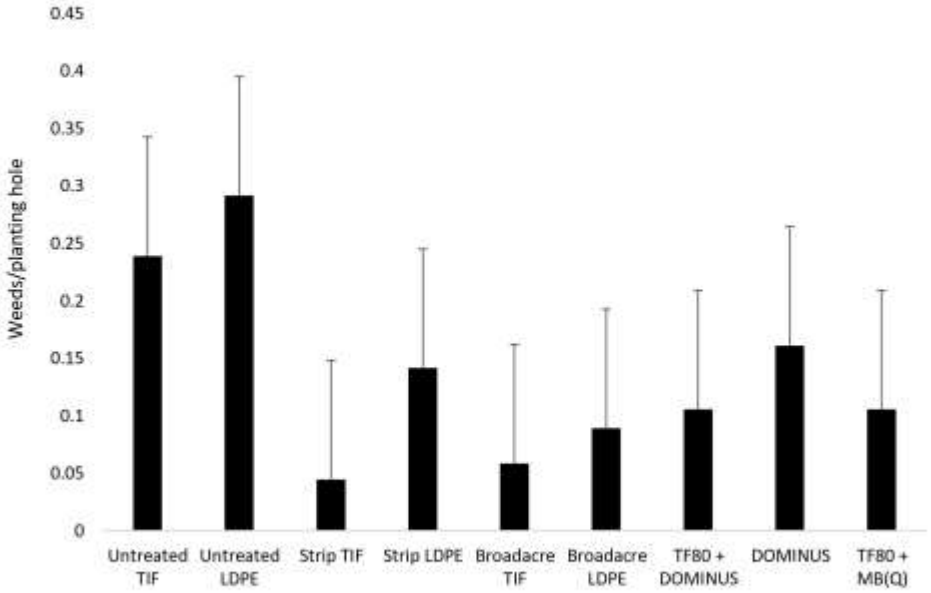

Figure 33. The number of weeds that emerged per strawberry planting hole, in a field trial at Silvan, Victoria. Bars represent LSDs where p = 0.05.

5.2.3.6. Fruit yields and revenue

Fumigant treatments significantly increased fruit production compared with the untreated controls (Table 6). Strip TIF, Broadacre LDPE and Broadacre TIF treatments significantly increased fruit production compared with the industry standard Strip LDPE.

There was no significant difference between the plugs and bare-rooted transplants, regarding their cumulative total fruit yield (average of 1430g/plant and 1421g/plant, respectively), marketable fruit yield (average of 834g/plant and 854g/plant, respectively), total number of fruit (average of 87/plant and 85/plant, respectively), marketable number of fruit (average of 47/plant and 46/plant, respectively) and fruit revenue (average of $4.75/plant and $4.82/plant, respectively). However, plugs generated significantly less profit than bare-rooted transplants in trial, due to their increased cost ($0.60 more costly). The average fruit size from plants grown from bare-rooted transplants (18.6g/piece) was significantly larger than from those grown from plugs (17.7g/piece) by 5%, throughout the study.

Table 6. Cumulative strawberry fruit production per plant from the 23/04/2018-2/01/2020 in a trial at Silvan, Victoria. The ordinal symbols represent significant differences, where p = 0.05, in each column. The costs associated with the treatments (e.g. fumigation) have not been deducted from the marketable fruit revenue data. The least significant difference (LSD) values are provided.

Treatment	Total fruit yield (g)	Marketable fruit yield (g)	Total number of fruit	Marketable number of fruit	Marketable fruit size (g/berry)	Marketable revenue (AU$)
Untreated TIF	870.92^{a}	533.79^{a}	52.23^{a}	29.22^{a}	18.37^{ab}	2.94^{a}
Untreated LDPE	939.18^{a}	551.8^{a}	56.7^{a}	30.35^{a}	18.41^{ab}	3.09^{a}
Strip TIF	1686.93^{d}	986.97^{e}	103.27^{cd}	55.95^{e}	17.6^{ab}	5.55^{c}
Strip LDPE	1213.2^{b}	709.32^{b}	73.63^{b}	38.17^{b}	18.59^{ab}	4.04^{b}
Broadacre TIF	1680.82^{d}	1005.32^{e}	98.37^{cd}	53.93^{de}	18.73^{b}	5.73^{c}
Broadacre LDPE	1729.99^{d}	972.69^{e}	103.03^{cd}	52.18^{cde}	18.65^{b}	5.65^{c}
TF80 + DOMINUS	1579.2^{cd}	964.12^{de}	94.95^{c}	53.08^{cd}	18.11^{ab}	5.468^{c}
DOMINUS	1377.88^{bc}	831.88^{c}	86.18^{bc}	47.58^{c}	17.45^{a}	4.69^{b}
TF80 + MB(Q)	1403.4^{c}	852.02^{cd}	85.93^{bc}	48.17^{cd}	17.7^{ab}	4.81^{b}
LSD	189.2	113.83	11.6	6	1.14	0.63

5.2.4. Conclusions

(a) Strip application of TF80 under TIF significantly reduced concentrations of *M. phaseolina* DNA in soil by 65% and charcoal rot by 68%, compared with application under LDPE. After accounting for the cost of TIF compared with LDPE, strip TIF increased profits from fruit by AU$8.97/m compared with strip LDPE.

(b) Broadacre LDPE reduced concentrations of *M. phaseolina* DNA in the soil by 57% and charcoal rot by 44% compared with strip LDPE fumigation. Broadacre fumigation with LDPE significantly increased marketable fruit yields and revenue compared with strip LDPE by 37% and 41%, respectively. Moreover, after accounting for the increased cost of treating a field with broadacre fumigation, broadacre TIF and broadacre LDPE increased income from fruit by AU$9.22/m and AU$8.93/m respectively, compared with strip LDPE.

(c) *M. phaseolina* was rarely detected in soils treated with TF80 + DOMINUS®, but was when TF80 and DOMINUS® were applied alone. Despite this, DOMINUS® co-applied with TF80 did not increase the marketable fruit yield or revenue from fruit, compared with TF80 applied alone.

(d) Since DOMINUS® + TF80 resulted in reduced *M. phaseolina* concentrations in soils compared with TF80 alone, there may be potential in combinations of biofumigant products and other non-fumigant treatments with fumigants to control charcoal rot. Therefore, further research into other non-chemical treatments, including anerobic soil disinfestation, microwave technologies, biofumigation and biocontrol products, to improve the management of charcoal rot is warranted.

5.3. Field experiment 3: Effectiveness of crop termination with metham sodium for controlling *M. phaseolina* in old strawberry crops

Location: Silvan, Victoria

Crop termination treatment: 13/2/2018

Final measurement: 01/4/2018

5.3.1. Background

Crop termination is the process of killing an old strawberry crop before preparing soil for a new planting. Crop termination with the fumigant metham sodium through the trickle line has the potential advantage of killing inoculum of *M. phaseolina* in the soil and residues of the old crop. Crop termination with metham sodium is practiced in California, USA for management of charcoal rot.

5.3.2. Aims

To determine if crop termination could be achieved, under field conditions in Australia, with the use of metham sodium, applied through the irrigation system.

Design: The experiment was a randomised complete block design. There were three plots of each treatment. Individual blocks were 10m long with 1.5m centres.

List of treatments

- Treated with metham sodium (100 L/ha) under LDPE, drip fumigation (based on rates used in the USA).
- Untreated.

5.3.3. Amended methods

- Fumigant concentrations were recorded in the soil after treatment.
- The crop was visually inspected for plant death seven days after treatment.

5.3.4. Results

5.3.4.1. Fumigant concentrations

We detected methyl isothiocyanate (active degradation product of metham sodium) in the centre and sides of the treated beds. Concentrations peaked at 100ppm, three hours post treatment in the centre of the beds.

5.3.4.2. Crop destruction

The crop did not die, within 7 days, post application of metham sodium.

5.3.5. Conclusions

(a) Crop terminations was not successful in this trial because there was insufficient plant death of the old strawberry crop 7 days after treatment.

(b) We therefore hypothesised that the application rates used in the USA for crop termination are too low for use in heavier soil types in Australia.

(c) Based on these results, the application rate of metham sodium in the following field trial was increased with the aim of improving its effectiveness for crop termination (see section 5.4.).

(d) Other soil disinfestation practices (e.g. biofumigant oils and microwave technologies) also have potential for crop termination, and these methods warrant further investigation.

5.4. Field experiment 4: Integrated use of crop termination with metham sodium and soil fumigation for control of charcoal rot

Location: Silvan, Victoria

Crop termination treatment: 22/2/2018

Soil fumigation: 11/01/2019

Planting: 13/02/2019

First Fruiting Period: 21/05/2019 – 11/06/2019

Second Fruiting Period: 01/09/2019 – 1/06/2020

5.4.1. Aims

To determine the effectiveness of (a) the integrated use of crop termination with metham sodium and pre-plant fumigation, and (b) co-application of the pre-plant fumigants Tri-Form® 80 with DOMINUS® for controlling charcoal rot.

5.4.2. Design

The experiment was a randomised split-plot design (Figure 34). Crop termination treatments formed the main plots (2 levels: metham sodium and untreated) and fumigant treatments formed the split plots (4 levels: Tri-Form®80, DOMINUS®, Trif-Form®80 co-applied with DOMINUS® and untreated). There were four blocks of each treatment. Individual plots were 6m long with 1.5m centres.

Figure 34. Field trial 4, Silvan, Victoria. (April 2019).

List of treatments (All treatments were planted with 30 bare-rooted transplants (cv. Albion)

- TF80 - Tri-Form® 80 (400 kg/ha) under TIF, shank fumigation.
- DOMINUS - DOMINUS® (200 L/ha) under TIF, sprayed and incorporated with a rotary hoe to 25 cm.
- TF80 + DOMINUS - Tri-Form® 80 (400 kg/ha), shank fumigated + DOMINUS® (200 L/ha) sprayed and incorporated with a rotary hoe to 25 cm, under TIF.
- Untreated - Untreated, under TIF.
- Termination TF80 - Crop termination (metham sodium (400 L/ha)) + Tri-Form® 80 under TIF, shank fumigation.
- Termination DOMINUS - Crop termination (metham sodium (400 L/ha)) + DOMINUS® (200 L/ha) under TIF, sprayed and incorporated with a rotary hoe to 25 cm.
- Termination TF80 + DOMINUS - Crop termination (metham sodium (400 L/ha)) + Tri-Form® 80 (400 kg/ha) shank fumigated, under TIF + DOMINUS® (200 L/ha), sprayed and incorporated with a rotary hoe to 25 cm.
- Termination Untreated - crop termination (treated with metham sodium (400 L/ha) under LDPE, drip fumigation).

5.4.3. Amended methods

- Fumigant concentrations were recorded after crop termination had occurred.

- The crop was visually inspected for plant death seven days after treatment. Plant samples were collected from the non-treated and treated samples to assess the viability of *M. phaseolina* inside the crowns.
- Fumigant concentrations were not recorded during this trial, post fumigation.

5.4.4. Results

5.4.4.1. Fumigant concentrations for crop termination

We detected metham sodium in the centre and sides of the treated beds. Concentrations peaked at 3000ppm, one hour after treatment in the centre of the beds.

5.4.4.2. Crop destruction

The old strawberry crop died by 7 days after treatment with metham sodium. There was no significant difference in the incidence of *M. phaseolina* inside the crowns of strawberry in the crop terminated (35.5%) and control (19%) treatments.

5.4.4.3. Pathogen viability in crowns

Tri-Form® 80 + DOMINUS® and TF80 alone significantly reduced the survival of *M. phaseolina* inside buried crowns by 100% compared with the control. DOMINUS® did not reduce the viability of *M. phaseolina* in buried crowns.

5.4.4.4. *M. phaseolina* concentrations in soil

On average, crop termination with metham sodium did not significantly reduce concentrations of *M. phaseolina* in the soil compared with the untreated control. All pre-plant fumigants reduced the concentration of *M. phaseolina* in the soil compared with the untreated controls (Table 7). Co-application of DOMINUS® with Tri-Form® 80 did not significantly increase pathogen control compared with Tri-Form® 80 alone. There was no significant interaction between crop termination and pre-plant fumigation in affecting *M. phaseolina* in soil.

Table 7. The average concentrations of *M. phaseolina* log DNA copies/g of soil in a field trial at Silvan, Victoria. Soil samples were collected at depths of 0-10cm from February 2019-June 2020. The ordinal symbols represent significant differences, where p = 0.05, in each column. The least significant difference (LSD) values are provided.

Treatment	**Date**						
	15/02/19	24/06/19	5/012/19	29/01/20	27/02/20	15/04/20	11/06/20
TF80	0^{a}	0^{a}	0^{a}	1.61^{a}	0^{a}	0^{a}	0.72^{a}
DOMINUS	0.62^{a}	0.62^{a}	3.1^{c}	1.8^{a}	3.55^{b}	0^{a}	1.45^{a}
TF80 + DOMINUS	0.59^{a}	0.59^{a}	0.59^{ab}	0.77^{a}	0^{a}	0^{a}	0.61^{a}
Untreated	3.54^{b}	4.3^{b}	3.84^{c}	3.88^{b}	2.93^{b}	3.7^{b}	3.94^{b}
Termination TF80	0.69^{a}	0.81^{a}	0.78^{ab}	0.86^{a}	0.69^{a}	0^{a}	0.92^{a}
Termination DOMINUS	0^{a}	0^{a}	0^{a}	0.92^{a}	2.46^{b}	0.79^{a}	0^{a}
Termination TF80 + DOMINUS	0^{a}	1.36^{a}	1.56^{b}	0.88^{a}	0.83^{a}	0.8^{a}	0.71^{a}
Termination Untreated	3.15^{b}	3.1^{b}	3.35^{c}	3.92^{b}	3.33^{b}	3.3^{b}	3.51^{b}
LSD	1.2	1.47	1.24	1.9	1.6	1.59	1.21

5.4.4.5. Charcoal rot incidence and severity

On average, crop termination with metham sodium did not significantly affect charcoal rot in the following crop. All fumigant treatments significantly reduced disease compared with the controls, except for DOMINUS® (Figure 35).

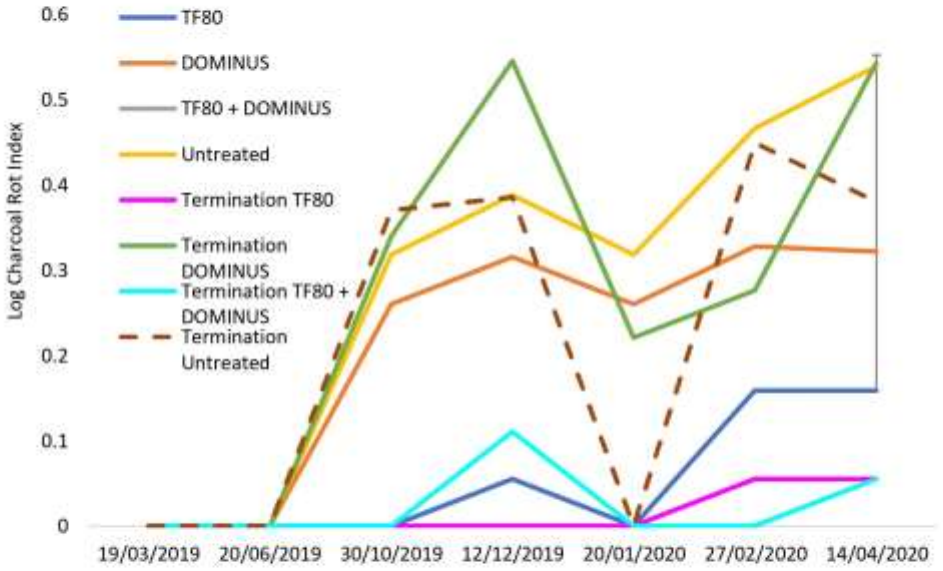

Figure 35. The index of charcoal rot in strawberry grown in different treatments in a field trial at Silvan, Victoria. Disease assessments were conducted at regular intervals from March 2019-April 2020. Bars represent LSDs where p = 0.05.

5.4.4.6. Weed assessments

There were significantly less weeds present in the plots that did not receive crop termination by 50%. There were no significant differences in weed emergence between the pre-plant fumigant treatments (Figure 36).

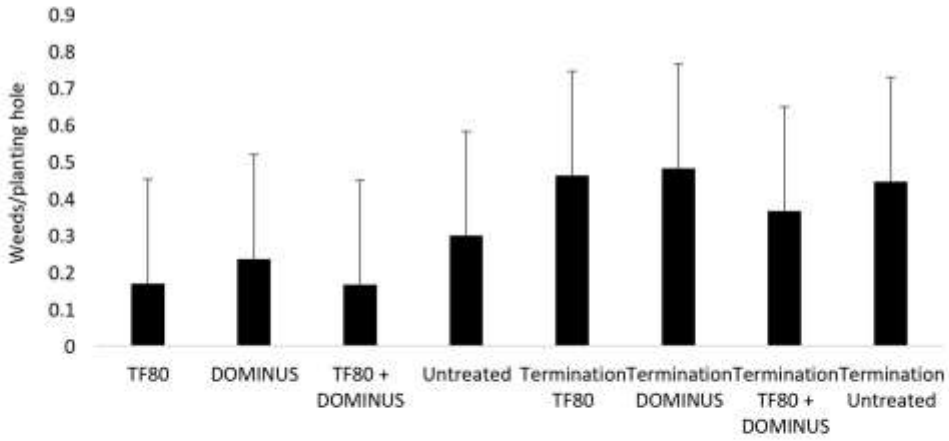

Figure 36. The number of weeds that emerged per strawberry planting hole, in a field trial at Silvan, Victoria. Bars represent LSDs where p = 0.05.

5.4.4.7. Fruit yields and revenue

The integration of crop termination with metham sodium followed by pre-plant fumigation significantly increased cumulative total fruit yield (by 24%), marketable fruit weight (by 30%), total number of fruit (by 21%), marketable number of fruit (by 29%) and revenue form fruit (by AU$1.29), compared with the pre-plant fumigation alone. Moreover, once you deduct the cost of the metham sodium treatment ($0.032/plant) there is still a significant difference in income between the two treatment groups.

The treatments that received crop termination with metham sodium, followed by pre-plant fumigation with DOMINUS® or DOMINUS® + TF80 increased fruit production compared with the untreated control (Table 8).

Table 8. Cumulative strawberry fruit production per plant from the 21/05/2019-4/06/2020, on a farm in Silvan, Victoria. The ordinal symbols represent significant differences, where p = 0.05, in each column. The costs associated with the treatments (e.g. fumigation) have not been deducted from the marketable fruit revenue data. The least significant difference (LSD) values are provided.

Treatment	Total fruit yield (g)	Marketable fruit yield (g)	Total number of fruit	Marketable number of fruit	Marketable fruit size (g/berry)	Marketable fruit revenue (AU$)
TF80	957.6^{b}	485^{ab}	54.8^{abc}	26.5^{abc}	18.3^{a}	3^{ab}
DOMINUS	840.8^{ab}	424.9^{ab}	50.5^{ab}	23.9^{ab}	18^{a}	2.7^{ab}
TF80 + DOMINUS	968.8^{b}	491.58^{bc}	53.7^{abc}	26.4^{abc}	18.7^{a}	3.1^{b}
Untreated	707.3^{a}	359.4^{a}	44^{a}	20.5^{a}	17.5^{a}	2.2^{a}
Termination TF80	1155.8^{bc}	612.8^{cd}	64.1^{cd}	32.7^{cd}	18.8^{a}	4^{cd}
Termination DOMINUS	1170.8^{bc}	701.5^{d}	66.2^{cd}	35.7^{d}	19.8^{a}	4.3^{d}
Termination TF80 + DOMINUS	1269^{c}	685^{d}	68.6^{d}	36.7^{d}	18.7^{a}	4.4^{d}
Termination Untreated	1016^{b}	543.1^{bc}	60.3^{bc}	30.4^{bcd}	17.9^{a}	3.4^{bc}
LSD	214.46	127.46	12.87	7.2	ns	0.8

5.4.5. Conclusions

(a) Crop termination with metham sodium applied at 400L/ha was successful in killing the old strawberry crop within seven days of treatment.

(b) Crop termination increased strawberry yields and revenue from fruit in the following crop, but this was not related to increased control of weeds, *M. phaseolina* or charcoal rot.

(c) The fumigated treatments (those treated with DOMINUS® and/or Tri-Form® 80) outperformed the untreated controls, regarding their fruit production and control of *M. phaseolina* in the soil.

(d) The co-application of Tri-Form® 80 and DOMINUS® did not improve the efficacy of either product applied alone, regarding the control of charcoal rot. However, increased application rates may improve the effectiveness of DOMINUS® for control of charcoal rot in a similar manner to metham sodium (note: both products hydrolyse to form isothiocyanate actives).

(e) Further research is required to understand the mechanism (possibly beneficial changes in soil biology) by which crop termination with metham sodium increases strawberry yields.

5.5. Field experiment 5: Comparative efficacy of ethanedinitrile and Tri-Form® 80 for control of charcoal rot

Location: Wandin, Victoria

Fumigated: 21/4/2018

Planted: 6/06/2018

Fruiting Period: 26/10/2018 – 11/06/2019

5.5.1. Aims

To compare: (a) the effectiveness of Tri-Form® 80 (TF80) and ethanedinitrile (EDN), and (b) the effect of crown size of bare-rooted transplants, on charcoal rot.

5.5.2. Design

The experiment was a randomised split-plot design (Figure 37). Fumigant treatments formed the main plots (3 levels: ethanedinitrile, Tri-Form® 80, and untreated) and transplant sizes formed the split plots (3 levels: small, medium and large). There were three plots of each treatment. Individual plots were 4m long with 1.5m centres.

Figure 37. Field trial 5, Silvan, Victoria. (January 2019).

List of fumigant treatments

- EDN - EDN (500 kg/ha), TIF, shank injected.
- TF80 - Tri-Form® 80 (400 kg/ha), TIF, shank injected.
- Untreated.

List of planting material treatments (All treatments were planted with 10 bare-rooted transplants (cv. Albion))

- Small - Bare-rooted transplants with small crowns (<9mm) (cv. Albion).
- Medium - Bare rooted transplants with medium crowns (9-12mm) (cv. Albion).
- Large - Bare rooted transplants with large crowns (>12mm) (cv. Albion)

5.5.3. Amended methods

- Fumigant concentrations were not measured in this trial.

5.5.4. Results

5.5.4.1. Pathogen viability in crowns

The fumigated treatments reduced the average viability of *M. phaseolina* inside strawberry crowns compared with the untreated control, though the difference was not statistically significant (Figure 38).

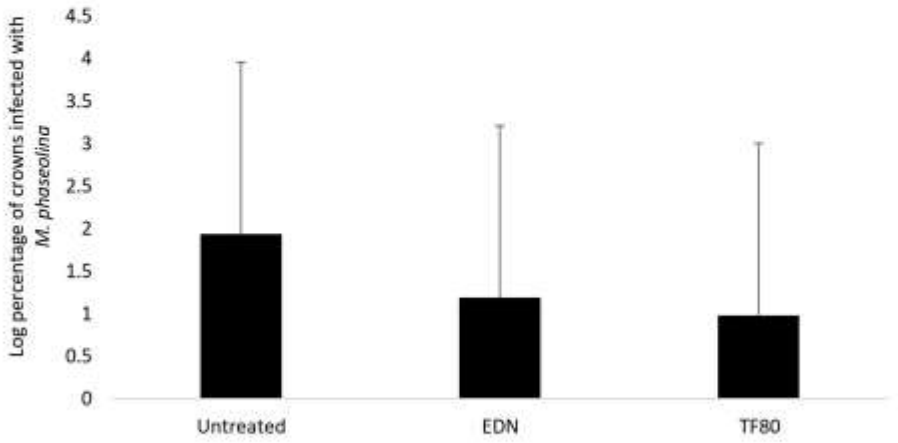

Figure 38. Log percentage of strawberry crowns infected with *M. phaseolina*, post fumigation, in a field trial at Wandin, Victoria. Bars represent LSDs where p = 0.05.

5.5.4.2. *M. phaseolina* concentrations in soil

The EDN and Tri-Form® 80 treatments significantly reduced the concentrations of *M. phaseolina* in soils compared with the untreated control, at the end of the study (Table 9). The concentrations of *M. phaseolina* in soils treated EDN and Tri-Form® 80 were not significantly different.

Table 9. The average concentrations of *M. phaseolina* log DNA copies/g of soil in a field trial at Wandin, Victoria. Soil samples were collected at depths of 0-10cm from May 2018 - May 2019. The ordinal symbols represent significant differences, where p = 0.05, in each column. The least significant difference (LSD) values are provided.

Treatment	**Date**						
	22/5/2018	29/8/2018	30/11/2018	12/12/2018	17/1/2019	14/3/2019	20/5/2019
Untreated	3.47^{b}	3.35^{b}	2.06^{a}	3.17^{b}	3.26^{b}	3.44^{b}	3.81^{b}
EDN	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	2.43^{ab}	0^{a}
TF80	0^{a}	0^{a}	0^{a}	0^{a}	0.9^{a}	0^{a}	0^{a}
LSD	0.25	0.65	2.33	0.14	2.17	2.88	0.042

5.5.4.3. Charcoal rot incidence and severity

Crown size of transplants did not impact disease at any point through the trial (Figure 39). Compared with the control, EDN and Tri-Form® 80 significantly reduced charcoal rot in strawberry plants, by 96% and 71% respectively. There was no significant difference in disease control between the EDN and Tri-Form® 80 treatments.

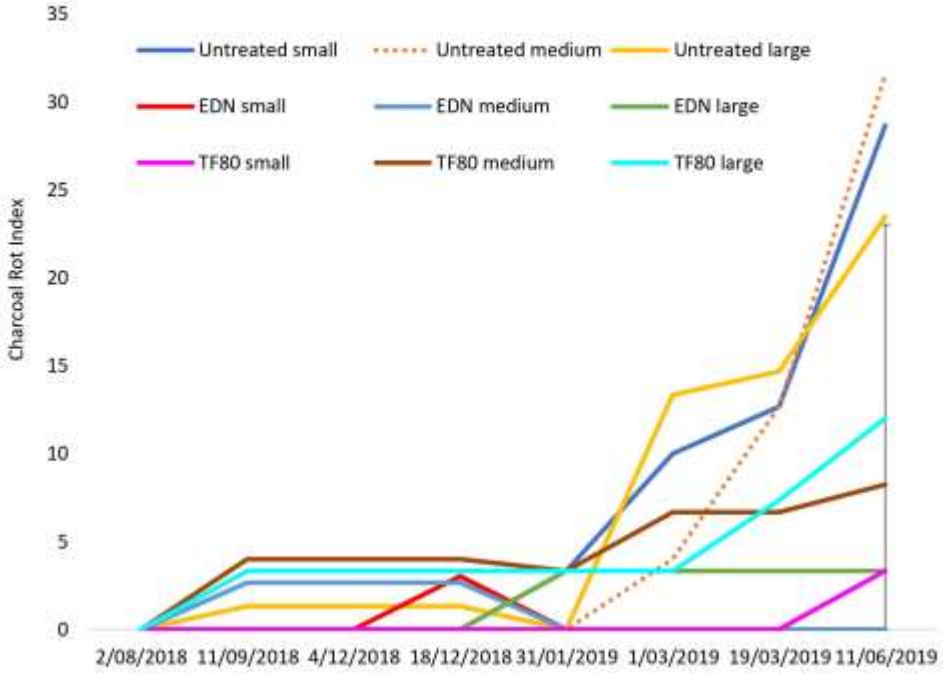

Figure 39. The index of charcoal rot in strawberry grown in different treatments in a field trial at Wandin, Victoria. Disease assessments were conducted at regular intervals from August 2018-June 2019. Bars represent LSDs where p = 0.05.

5.5.4.4. Weed assessments

The fumigated treatments (Tri-Form® 80 and EDN) significantly reduced weed emergence compared with the untreated control (by 85% and 66%, respectively) (Figure 40).

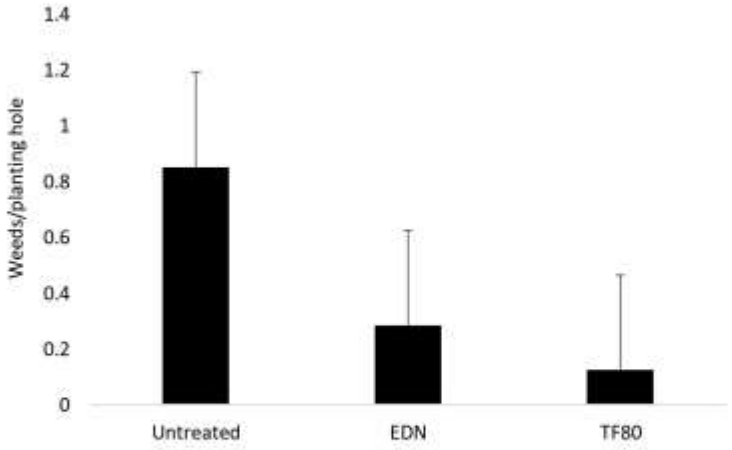

Figure 40. The number of weeds that emerged per strawberry planting hole, in a field trial at Wandin, Victoria. Bars represent LSDs where p = 0.05.

5.5.4.5. Fruit yields and revenue

Crown size did not impact fruit production (Table 10). Fumigation with EDN and Tri-Form® 80 significantly increased marketable fruit yields by 38% and revenue from fruit by $1.13/plant compared with the untreated control (Table 11). There was no significant difference between the Tri-Form® 80 and EDN treatments, regarding fruit production.

Table 10. Cumulative strawberry fruit production per plant from the 26/10/2018 to the 11/6/2019, on a farm in Wandin, Victoria. The ordinal symbols represent significant differences, where p = 0.05, in each column. The least significant difference (LSD) values are provided.

Treatments	Total fruit yield (g)	Marketable fruit yield (g)	Total number of fruit	Marketable number of fruit	Marketable fruit size (g/berry)	Marketable revenue (AU$)
Small transplants (<9mm)	914[a]	495.18[a]	49.93[a]	23.79[a]	20.73[a]	3.12[a]
Medium transplants (9 – 12mm)	940[a]	495.08[a]	52.26[a]	25.14[a]	20.15[a]	3.15[a]
Large transplants (>12mm)	988[a]	494.52[a]	55.21[a]	24.52[a]	20.08[a]	3.02[a]
LSD	ns	ns	ns	ns	ns	ns

Table 11. Cumulative strawberry fruit production per plant from the 26/10/2018 to the 11/6/2019, on a farm in Wandin, Victoria. The ordinal symbols represent significant differences, where p = 0.05, in each column. The costs associated with the treatments (e.g. fumigation) have not been deducted from the marketable fruit revenue data. The least significant difference (LSD) values are provided.

Treatments	Total fruit yield (g)	Marketable fruit yield (g)	Total number of fruit	Marketable number of fruit	Marketable fruit size (g/berry)	Marketable revenue (AU$)
Untreated	743[a]	376.27[a]	43.1[a]	19.14[a]	19.83[a]	2.2[a]
EDN	962[b]	525.72[b]	54.08[b]	26.52[b]	20.1[a]	3.33[b]
TF80	1136[c]	582.78[b]	60.22[b]	27.79[b]	21.03[a]	3.45[b]
LSD	169.2	99.11	9.26	6.14	ns	7.07

5.5.5. Conclusions

(a) The crown size of bare-rooted transplants did not impact charcoal rot or fruit yields of mature strawberry plants.

(b) EDN and Tri-Form® 80 were equally effective in reducing the viability of *M. phaseolina* in infected strawberry crowns, the concentrations of *M. phaseolina* DNA in the soil, and charcoal rot incidence and severity, compared with the untreated control.

(c) EDN and Tri-Form® 80 were equally effective in increasing the marketable yield of strawberry (by 39% and 54%, respectively) compared with the untreated control.

(d) EDN and Tri-Form® 80 did not control *M. phaseolina* in strawberry crowns in the soil in this trial. Therefore, *M. phaseolina* surviving in infested crowns may recolonise treated soils and form a source of inoculum of new plants. Further work is required on integrating fumigation with cultural practices of removing old crowns from soil or making old crowns more conducive to treatment with fumigants (e.g. crop termination treatments).

5.6. Field experiment 6: Effectiveness of co-application of Tri-Form® 80 and ethanedinitrile under different coloured totally impermeable films (TIFs) for control of charcoal rot

Location: Coldstream, Victoria

Fumigated: 10/04/2018

Planted: 10/05/2018

Fruiting Period: 19/10/2018 – 30/01/2020

5.6.1. Aims

To compare: (a) the effectiveness of co-application of Tri-Form® 80 (TF80) with EDN for controlling charcoal rot of strawberry, and (b) the effect of the colour of TIF film (silver or black) and soil temperature (measured with dataloggers) on the expression of charcoal rot of strawberry.

5.6.2. Design

The experiment was a randomised complete block design (Figure 41). There were three blocks of each treatment. Individual blocks were 4m long with 1.5m centres.

Figure 41. Field trial 6, Coldstream, Victoria. (January 2019).

List of fumigant treatments (All treatments were planted with 20 bare-rooted transplants (cv. Albion))

- TF80 black - Tri-Form® 80 (400 kg/ha) shanked, under black TIF.
- TF80 silver - Tri-Form® 80 (400 kg/ha) shanked, under silver TIF.
- TF80 + EDN black - Tri-Form® 80 (400 kg/ha) shanked + EDN (500 kg/ha) drip fumigation, under black TIF.

- TF80 + EDN silver - Tri-Form® 80 (400 kg/ha) shanked + EDN (500 kg/ha) drip fumigation, under silver TIF.
- Untreated – untreated soil under black TIF.

5.6.3. Amended methods

- Fumigant concentrations were not measured in this trial.
- Pathogen viability assays were not conducted in this trial.
- Temperature dataloggers (TInyTag®) were inserted into the centres of strawberry beds at depths of 15cm to compare black TIF, silver TIF and ambient air temperatures.

5.6.4. Results

5.6.4.1. *M. phaseolina* concentrations in soil

here was no significant difference in the concentration of *M. phaseolina* in soil between fumigant treatments (Tri-Form® 80 + EDN or Tri-Form® 80 alone) or the colour of the TIF, across the study period (Table 12).

Table 12. The average concentrations of *M. phaseolina* log DNA copies/g of soil in a field trial at Coldstream, Victoria. Soil samples were collected at depths of 0-10cm from October 2018 - November 2019. The ordinal symbols represent significant differences, where p = 0.05, in each column. The least significant difference (LSD) values are provided.

Treatment	**Date**						
	18/10/18	28/11/18	12/12/18	17/1/19	7/3/19	17/5/19	7/11/19
Untreated	0^{a}	0.97^{a}	0^{a}	2.17^{ab}	1.03^{a}	2.11^{ab}	nd
TF80 black	1.75^{b}	1.99^{a}	0.86^{a}	0^{a}	0.77^{a}	0^{a}	3.52^{ab}
TF80 silver	3.15^{c}	3.7^{a}	3.44^{b}	3.4^{b}	3.62^{b}	2.39^{b}	3.83^{ab}
TF80 + EDN black	0.77^{ab}	0.93^{a}	0.87^{a}	1.14^{a}	0.97^{a}	1.07^{ab}	4.19^{b}
TF80 + EDN silver	0^{a}	0.86^{a}	1.03^{a}	0.89^{a}	0^{a}	0^{a}	2.01^{a}
LSD	1.67	2.95	2.29	2.23	2.28	2.37	1.851

Nd = no data.

5.6.4.2. Charcoal rot incidence and severity

All fumigant treatments significantly reduced disease compared with the untreated control, except for Tri-Form® 80 under silver TIF (Figure 42). Black TIF significantly reduced disease, compared with silver TIF, by at least 80%, from the 19/3/2019 to the 11/6/2019. There was no significant difference in control of charcoal rot between the Tri-Form® 80 and Tri-Form® 80 +EDN treatments.

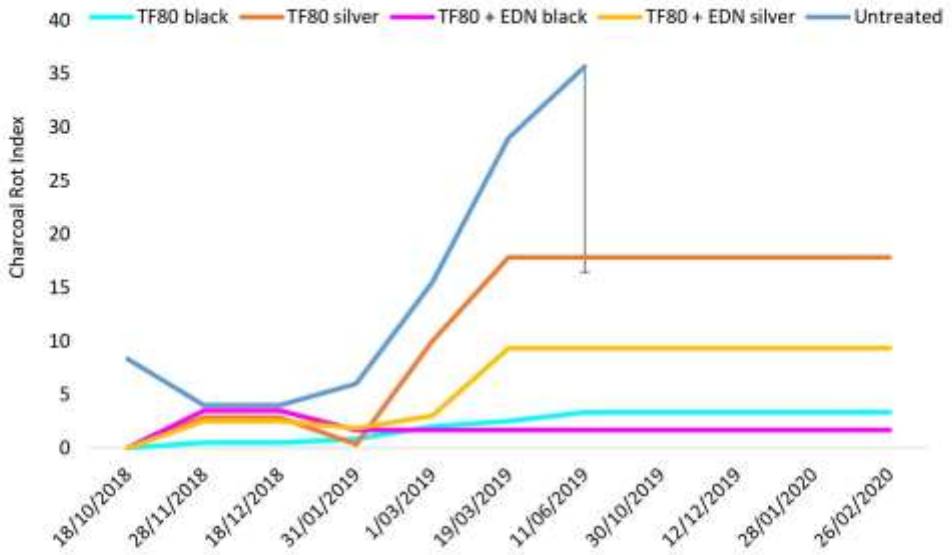

Figure 42. The index of charcoal rot in strawberry grown in different treatments in a field trial at Coldstream, Victoria. Disease assessments were conducted at regular intervals from October 2018 – February 2020. Bars represent LSDs where p = 0.05.

5.6.4.3. Soil temperature comparisons between silver and black TIF

The air temperature (15.99˚C) was, on average, significantly lower than the soil temperatures (17.86˚C). The soil temperatures under the silver TIF (17.96˚C) were, on average, higher than those under black TIF (17.77˚C) (p = 0.001) (Figure 43). The largest temperature fluctuation from their mean temperatures, for the black and silver TIF data sets, were 10.4˚C and 16.4˚C respectively.

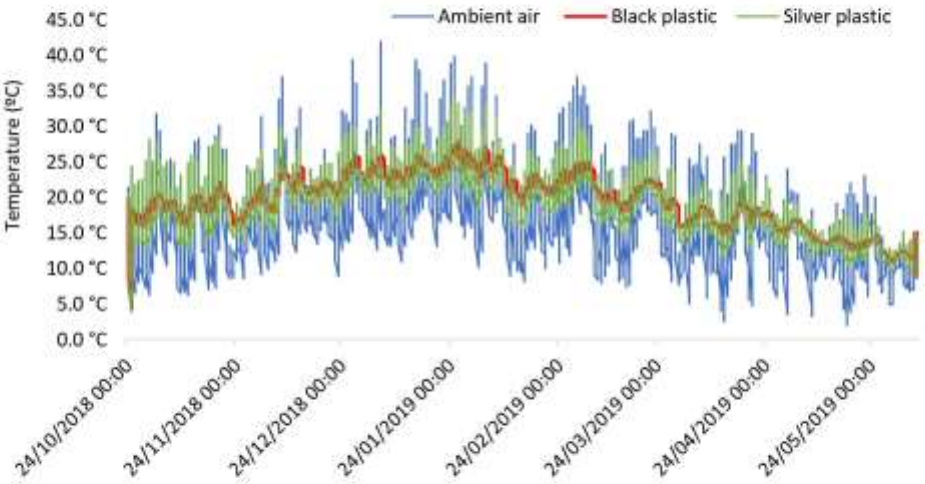

Figure 43. Average soil temperatures under black and silver TIF (15cm), at field trial in Coldstream, Victoria. Corresponding air temperatures (1m) are also displayed.

5.6.4.4. Weed assessments

Soil fumigation significantly reduced weed emergence compared with the untreated control by ~70% (Figure 44). There was no significant difference in weed emergence between fumigants (Tri-Form® 80 + EDN or Tri-Form® 80 alone) or the colour of the TIF.

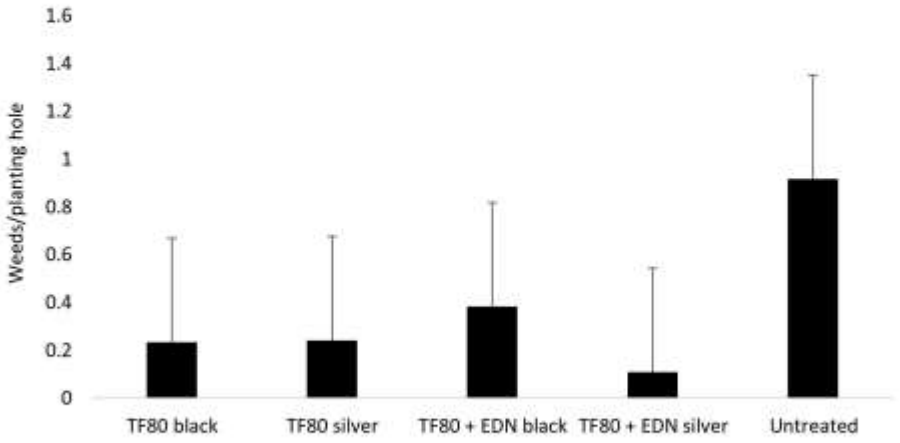

Figure 44. The number of weeds that emerged per strawberry planting hole, in a field trial at Coldstream, Victoria. Bars represent LSDs where p = 0.05.

5.6.4.5. Fruit yields and revenue

All fumigated treatments significantly increased fruit production compared with the untreated control (Table 13 & Figure 45). There was no significant difference between fumigants (Tri-Form® 80 + EDN or Tri-Form® 80 alone) or the colour of the TIF (silver or black), regarding their fruit production.

Table 13. Cumulative strawberry fruit production per plant from the 19/10/2018 to the 30/01/2020, on a farm in Coldstream, Victoria. The ordinal symbols represent significant differences, where p = 0.05, in each column. The costs associated with the treatments (e.g. fumigation) have not been deducted from the marketable fruit revenue data. The least significant difference (LSD) values are provided.

Treatments	Total fruit yield (g)	Marketable fruit yield (g)	Total number of fruit	Marketable number of fruit	Marketable fruit size (g/berry)	Marketable revenue (AU$)
Untreated*	389.7^{a}	178.9^{a}	28.2^{a}	11.7^{a}	14.5^{a}	1.03^{a}
TF80 black	1625.8^{b}	727.4^{b}	100.1^{b}	42.6^{b}	17^{ab}	4.27^{b}
TF80 silver	1442.6^{b}	677.5^{b}	93.3^{b}	38.9^{b}	17.5^{ab}	4.02^{b}
TF80 + EDN black	1392.2^{b}	630.1^{b}	91.1^{b}	36.3^{b}	17.4^{ab}	3.81^{b}
TF80 + EDN silver	1625^{b}	734.6^{b}	98^{b}	40.9^{b}	18^{b}	4.27^{b}
LSD	330.56	128.76	18.17	6.82	3.05	0.68

* The Untreated treatment was only picked until the 23/4/2019.

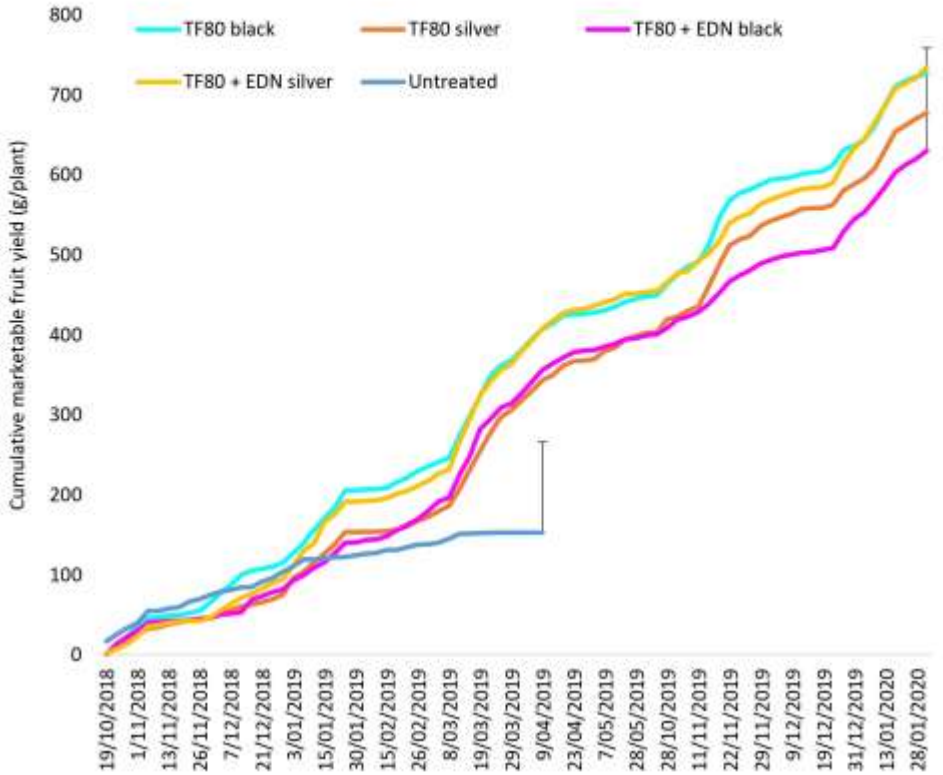

Figure 45. The cumulative marketable fruit yield/plant produced by strawberry on a farm in Coldstream, Victoria. Bars represent LSDs where p = 0.05.

5.6.5. Conclusions

(a) The fumigated treatments significantly reduced charcoal rot and weed emergence compared with the untreated control.

(b) Co-application of Tri-Form® 80 with EDN did not significantly improve control of *M. phaseolina* DNA in the soil, charcoal rot, weed emergence or increase fruit production compared with Tri-Form® 80 applied alone.

(c) The colour of TIF did not significantly affect the concentrations of *M. phaseolina* DNA in soils, weed emergence or fruit yield. The use of black TIF significantly reduced disease incidence by 80% compared with silver TIF, at the end of summer (March 2019).

(d) The reduction in charcoal rot under black TIF, compared with silver TIF, may be associated with less variability in soil temperatures under this treatment. Additional research should investigate the cause/s behind the differences in temperature variation under black and silver TIF (e.g. differences in canopy cover between treatments).

5.7. Field experiment 7: Impact of re-planting into fumigated beds on charcoal rot

Location: Coldstream, Victoria

Fumigated: 07/04/2017

First Planting: 19/05/2017

First Fruiting Period: 03/10/17 – 31/05/2018

Replanted with New Transplants: 01/05/2018

Second Fruiting Period: 23/08/2018 - 12/03/19

5.7.1. Background

Some strawberry growers have adopted the practice of replacing strawberry plants lost to charcoal rot in the first year of production, by planting new transplants into the same holes in the second year of production. This trial examined this practice in soils that were fumigated with different treatments in the first year of production.

5.7.2. Aims

To compare the effectiveness of Tri-Form® 80 and ethanedinitrile for control of charcoal rot in the second year of strawberry production.

5.7.3. Design

The experiment was a randomised complete block (Figure 46). There were three blocks of each treatment. Individual blocks were 5m long with 1.5m centres.

Figure 46. Field trial 7, Coldstream, Victoria. (January 2019).

List of treatments (All treatments were planted 40 bare-rooted transplants (cv. Albion))

- TF80 - Tri-Form® 80 (400 kg/ha), under TIF, shank injection.
- EDN - Ethanedinitrile (500 kg/ha), under TIF, shank injection.
- Untreated- untreated, under TIF.

5.7.4. Amended methods

- Assessments were made in the second year of production only.
- Fumigant concentrations were not measured in this trial.
- Pathogen viability assays were not conducted in this trial.
- Weed assessments were not conducted in this trial.

5.7.5. Results

5.7.5.1. *M. phaseolina* concentrations in soil

There were no significant differences in the concentrations in *M. phaseolina* in soil between the treatments (Table 14).

Table 14. The average concentrations of *M. phaseolina* log DNA copies/g of soil in a field trial at Coldstream, Victoria. Soil samples were collected at depths of 0-10cm from October 2018 - March 2019. The ordinal

symbols represent significant differences, where p = 0.05, in each column. The least significant difference (LSD) values are provided.

Treatment	Date				
	18/10/18	28/11/18	12/12/18	17/01/19	7/03/19
TF80	2.38[a]	3.85[a]	3.62[a]	3.64[a]	3.44[a]
EDN	3.02[a]	2.8[a]	2.04[a]	3.32[a]	4.31[a]
Untreated	3.43[a]	3.76[a]	3.81[a]	3.95[a]	4.23[a]
LSD	ns	ns	ns	ns	ns

5.7.5.2. Charcoal rot incidence and severity

The fumigated treatments significantly reduced disease compared with the untreated control from the 31/1/2019 to the 1/3/2019 (Figure 47). There was no significant difference in disease between the Tri-Form® 80 and EDN treatments.

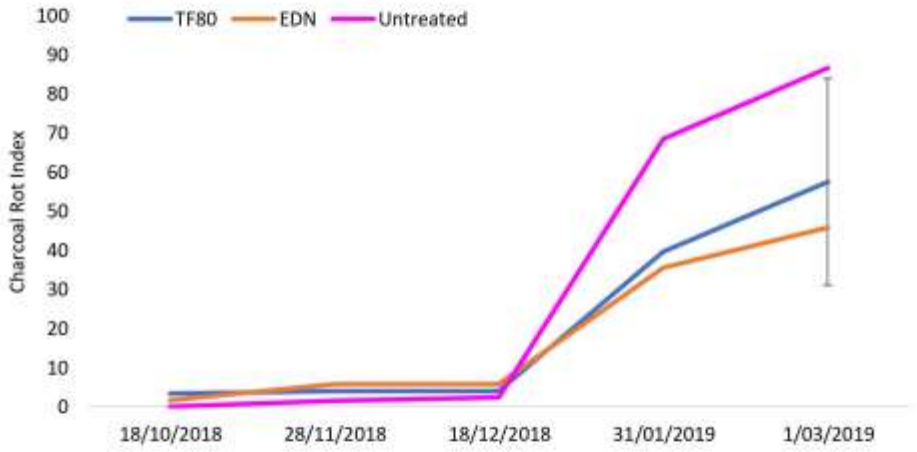

Figure 47. The index of charcoal rot in strawberry grown in different treatments in a field trial at Coldstream, Victoria. Disease assessments were conducted at regular intervals from October 2018 – January 2019. Bars represent LSDs where p = 0.05.

5.7.5.3. Fruit yields and revenue

Tri-Form® 80 significantly increased marketable strawberry yields by 85% compared with the control and by 55% compared with the EDN treatment (Table 15). Treatments with Tri-Form® 80 increased revenue from fruit by $0.27 per plant compared with the control. In contrast, EDN did not significantly increase revenue from fruit compared with the control.

Table 15. Cumulative strawberry fruit production per plant from the 23/08/2018-12/3/2019, on a farm in Coldstream, Victoria. The ordinal symbols represent significant differences, where p = 0.05, in each column. The costs associated with the treatments (e.g. fumigation) have not been deducted from the marketable fruit revenue data. The least significant difference (LSD) values are provided.

Treatment	Total fruit yield (g)	Marketable fruit yield (g)	Total number of fruit	Marketable number of fruit	Marketable fruit size (g/berry)	Marketable fruit revenue (AU$)
TF80	199^{b}	102.4^{b}	15.8^{b}	6.8^{b}	15.1^{a}	0.58^{b}
EDN	141.2^{ab}	66.5^{a}	12.7^{ab}	4.7^{ab}	14^{a}	0.4^{ab}
Untreated	102.4^{a}	55^{a}	7.7^{a}	3.4^{a}	16.1^{a}	0.31^{a}
LSD	64.8	35.51	5.46	2.35	ns	0.23

5.7.6. Conclusions

(a) Tri-Form® 80 significantly increased marketable fruit yields compared with EDN and the untreated control.

(b) The TF80 and EDN treatments did not differ, regarding their control of charcoal rot or *M. phaseolina* concentrations in soils.

(c) Re-planted transplants are not economically viable for commercial production. The cost of transplants is between AU$0.40 and $0.80. The best marketable fruit revenue per plant in this trial was $0.58. Therefore, the practice of replanting empty holes with transplants on paddocks that are affected by charcoal rot is not economically viable, because plants barely product enough revenue to cover the costs of the new transplants.

(d) Researchers in the USA are investigating the use of DOMUNIS® and metham sodium to refumigate beds, prior to replanting new transplants. The success of such a practice might be guided by diagnostic tests that predict disease.

5.8. Field experiment 8: Effectiveness of ethanedinitrile (EDN) and Tri-Form 80® (TF80) co-applied with dazomet for control of charcoal rot

Location: Coldstream, Victoria

Fumigated: 18/04/2019

Planted: 10/06/2019

Fruiting Period: 01/09/2019 – 1/06/2020

5.8.1. Aims

To determine if co-application of fumigants, including Dazomet (generator of methyl isothiocyanate), Agrocelhone FE (60% chloropicrin, 40% 1,3-dichloropropene) and ethanedinitrile, increases control of charcoal rot.

5.8.2. Design

The experiment was a randomised complete block design (Figure 48). There were four blocks of each treatment. Individual blocks were 4m long with 1.5m centres.

Figure 48. Field trial 8, Coldstream, Victoria. (February 2020).

List of treatments (All treatments were planted with 20 bare-rooted transplants (cv. Cabrillo))

- Untreated - Untreated under TIF.
- EDN - Ethanedinitrile (500 kg/ha) under TIF, shank fumigation.
- AGRO - Agrocelhone FE (400 kg/ha) under TIF, shank fumigation.
- Dazomet - Dazomet (300 kg/ha), spread on soil and incorporated to a depth of 25 cm with a rotary hoe, then covered with TIF.
- EDN + Dazomet - Ethanedinitrile (500 kg/ha) under TIF, shank fumigation + Dazomet (300 kg/ha), spread on soil and incorporated to a depth of 25 cm with a rotary hoe.
- EDN + AGRO - Ethanedinitrile (500 kg/ha) + Agrocelhone FE (400 kg/ha) under TIF, shank fumigation.
- AGRO + Dazomet - Agrocelhone FE (400 kg/ha) under TIF, shank fumigation + Dazomet (300 kg/ha), spread on soil and incorporated to a depth of 25 cm with a rotary hoe.
- EDN + AGRO + Dazomet - Ethanedinitrile (500 kg/ha) + Agrocelhone FE (400 kg/ha) under TIF, shank fumigation + Dazomet (300 kg/ha), spread on soil and incorporated to a depth of 25 cm with a rotary hoe.

5.8.3. Amended methods

- Fumigant concentrations were not measured during this trial.

5.8.4. Results

5.8.4.1. Pathogen viability in crowns

Soil fumigation significantly reduced the viability of *M. phaseolina* inside buried strawberry crowns compared with the untreated control, by at least 300% (Figure 49).

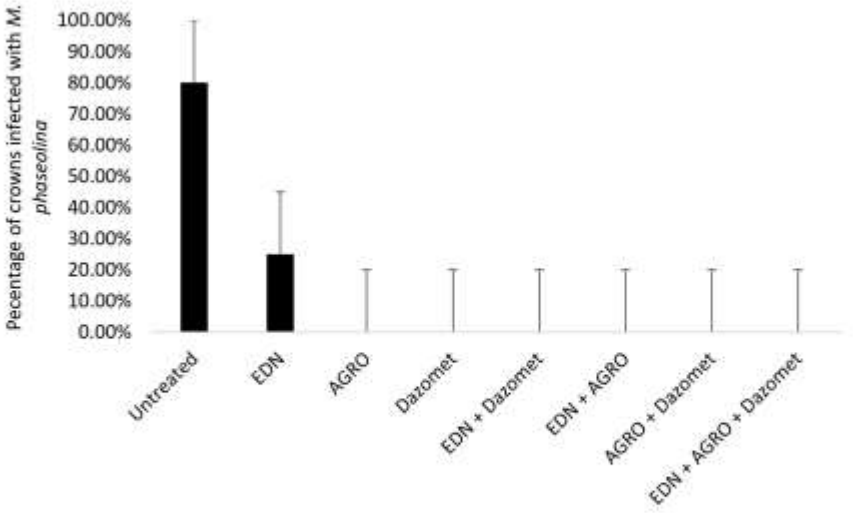

Figure 49. Percentage of strawberry crowns infected with *M. phaseolina*, post fumigation, in a field trial at Coldstream, Victoria. Bars represent LSDs where p = 0.05.

5.8.4.2. *M. phaseolina* concentrations in soil

Soil fumigation reduced the concentrations of *M. phaseolina* DNA in soils compared with the untreated control, throughout the study (Table 16). However, *M. phaseolina* gradually recolonised soils treated with dazomet. By the end the experiment, concentrations of *M. phaseolina* were not significantly different in soils treated with dazomet or the untreated control.

Table 16. The average concentrations of *M. phaseolina* log DNA copies/g of soil in a field trial at Coldstream, Victoria. Soil samples were collected at depths of 0-10cm from June 2019 - June 2019. The ordinal symbols represent significant differences, where p = 0.05, in each column. The least significant difference (LSD) values are provided.

Treatment	Date					
	24/06/19	22/11/19	29/01/20	27/02/20	15/04/20	11/06/20
Untreated	3.48^{c}	2.78^{b}	3.0^{b}	3.25^{b}	3.93^{c}	3.98^{c}
EDN	2.28^{bc}	0^{a}	0.77^{ab}	0^{a}	1.95^{b}	0.92^{a}
AGRO	0^{a}	0.64^{a}	0.76^{ab}	0^{a}	1.46^{ab}	1.59^{ab}
Dazomet	0^{a}	0^{a}	1.14^{ab}	1.52^{ab}	2.03^{b}	3.22^{bc}
EDN + Dazomet	0^{a}	0^{a}	1.39^{ab}	0^{a}	0^{a}	0.88^{a}
EDN + AGRO	1.59^{ab}	0^{a}	0^{a}	0.65^{a}	0^{a}	0.74^{a}
AGRO + Dazomet	0^{a}	0^{a}	1.64^{ab}	1.32^{ab}	0^{a}	1.01^{ab}
EDN + AGRO + Dazomet	0.61^{ab}	0.65^{a}	1.12^{ab}	0.65^{a}	0^{ab}	0^{a}
LSD	1.7	1.76	2.4	2.24	1.79	2.25

5.8.4.3. Charcoal rot incidence and severity

At the end of the trial, fumigated treatments, except for EDN + AGRO + Dazomet, significantly reduced charcoal rot compared with the untreated control, by at least 196% (Figure 50). It is possible that some of the plant deaths in the EDN + AGRO + Dazomet treatment were due to fumigant phytotoxicity and not charcoal rot. There was no significant difference in disease between any of the fumigated treatments, at the end of the trial.

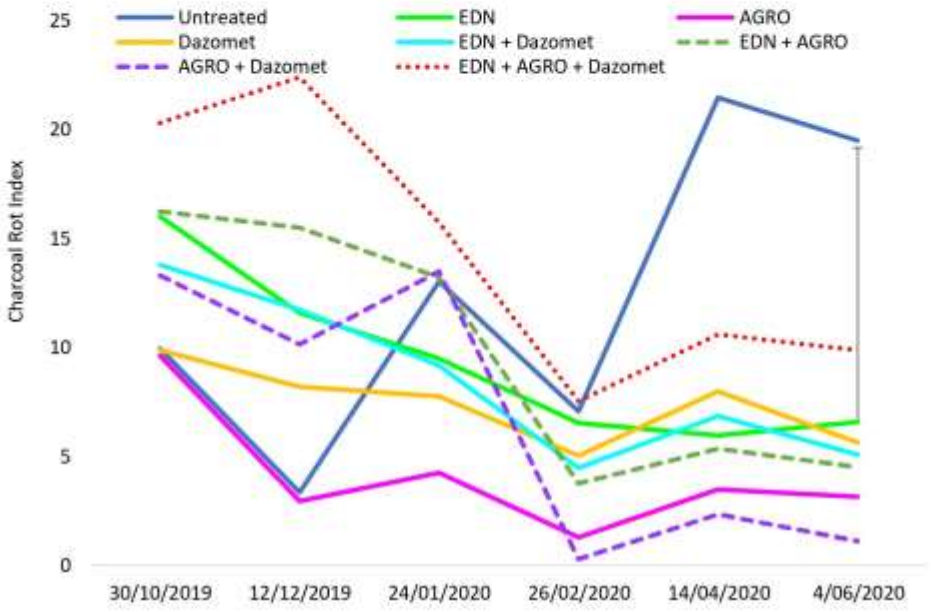

Figure 50. The index of charcoal rot in strawberry grown in different treatments in a field trial at Coldstream, Victoria. Disease assessments were conducted at regular intervals from October 2019 - June 2020. Bars represent LSDs where p = 0.05.

5.8.4.4. Weed assessments

Few weeds emerged across the trial site. EDN alone contained significantly more weeds than all other treatments, except for AGRO + Dazomet (Figure 51).

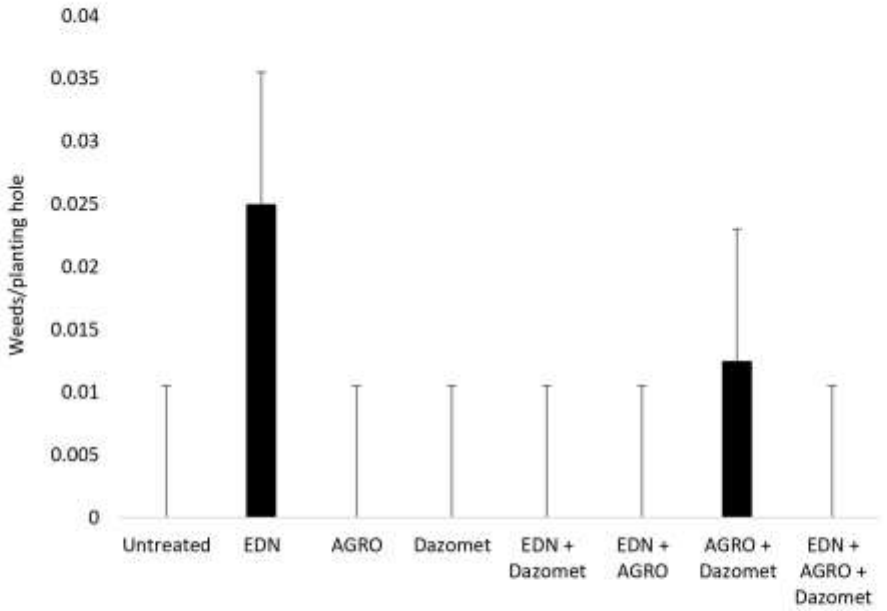

Figure 51. The number of weeds that emerged per strawberry planting hole, in a field trial at Coldstream, Victoria. Bars represent LSDs where p = 0.05.

5.8.4.5. Fruit yields and revenue

Agrocelhone FE alone and Dazomet alone significantly increased marketable fruit yields and fruit revenue compared with the untreated control (Table 17). Combinations of fumigants, including AGRO + Dazomet, EDN + Dazomet and EDN + AGRO, did not significantly increase fruit yields compared with either fumigant applied alone. Combinations of all three fumigants (EDN + AGRO + Dazomet) significantly reduced fruit yields compared with either fumigant applied alone. This was probably due to the effects of fumigant phytotoxicity from the combination of the three fumigants.

Table 17. Cumulative strawberry fruit production per plant from the 21/10/2019 - 4/6/2020, in a farm at Coldstream, Victoria. The ordinal symbols represent significant differences, where p = 0.05, in each column. The costs associated with the treatments (e.g. fumigation) have not been deducted from the marketable fruit revenue data. The least significant difference (LSD) values are provided.

Treatment	Total fruit yield (g)	Marketable fruit yield (g)	Total number of fruit	Marketable number of fruit	Marketable fruit size (g/berry)	Marketable fruit revenue (AU$)
Untreated	293.8^{a}	138.4^{ab}	21.4^{a}	7.6^{ab}	18.16^{ab}	1.04^{ab}
EDN	341.9^{ab}	151.7^{bc}	23.8^{ab}	8.5^{bc}	17.99^{ab}	1.12^{b}
AGRO	472.5^{d}	234.6^{e}	30.6^{d}	12^{d}	19.51^{c}	1.77^{d}
Dazomet	420.1^{cd}	202.2^{de}	27.3^{bc}	10.8^{d}	18.8^{abc}	1.55^{cd}
EDN + Dazomet	367.7^{bc}	153.1^{b}	26^{bc}	8.6^{bc}	17.68^{a}	1.15^{b}
EDN + AGRO	406.1^{c}	182.7^{cd}	26.51^{bc}	9.6^{bc}	19.02^{bc}	1.36^{bc}
AGRO + Dazomet	408.3^{c}	186^{cd}	27.7^{cd}	10.1^{cd}	18.29^{abc}	1.41^{bc}
EDN + AGRO + Dazomet	301.5^{a}	108^{a}	21.8^{a}	6.1^{a}	17.71^{ab}	0.78^{a}
LSD	63.48	40.3	3.85	2.05	1.4	0.31

5.8.5. Conclusions

(a) The fumigated treatments, except for EDN + AGRO + Dazomet, significantly reduced charcoal rot compared with the untreated control.

(b) Combinations of fumigants did not significantly improve fruit yields compared with either fumigant applied alone.

(c) A combination of all three fumigants (EDN + AGRO + Dazomet) was detrimental to the control of charcoal rot and fruit yields, compared with either fumigant applied alone.

(d) Future research should aim to develop more accurate plant-back times for fumigant combinations to avoid fumigant phytotoxicity.

6. Survival experiments

Two field studies were undertaken to determine the longevity of *M. phaseolina* within crowns, in sub-tropical (Sunshine Coast) and in the temperate (Granite Belt) growing environments of the Australian strawberry industry. The timing and duration of each study reflected the off-season in each growing environment.

M. phaseolina isolate used

M. phaseolina isolate BRIP 66625, was used in this study to inoculate healthy strawberry plants which were subsequently used as inoculum. It's pathogenicity on strawberry was demonstrated after a recent study in Queensland (Gomez et al., 2020). BRIP 66625 was originally isolated in the laboratory, using methods described by Hutton et al. (2013) from wilting strawberry plants (cv. Florida radiance) collected from a commercial farm in Glenview, Queensland in 2009. *M. phaseolina* was identified based on morphological growth on the plate and verified by DNA sequencing of the internal transcribed spacer region.

M. phaseolina inoculum suspension

Inoculum was prepared as per methods described by Gomez et al. (2020). Briefly, BRIP 66625 was revived from storage by sub-culturing onto quarter-strength potato dextrose agar (PDA) amended with 50 ppm streptomycin sulfate (Sigma-Aldrich, St. Louis, MO, USA) and incubated at 24° C in continuous near-UV light incubator. After three weeks, PDA with microsclerotia imbedded were blended with sterile deionised water (SDW) to produce a microsclerotial suspension. The concentration of the suspension was adjusted to 1.4×10^3 microsclerotia per mL.

Inoculation of strawberry plants to be used as buried inoculum source

Certified strawberry transplants cv. Albion were grown in pots with sterilised 1:1 mix of sand and peat. Plants were maintained in a greenhouse for up to 6 months and given regular watering and a standard nutrient program prior to inoculations. Plants were inoculated by pouring 50 mL of the microsclerotia inoculum suspension into the potting mix and transferred to an evaporatively cooled glasshouse on a heated bench set at 30° C. Plants with symptoms of wilt were observed from 6 weeks after inoculation. After 12 weeks, infected crowns were collected and prepared (washed, trimmed, and cut in half) as inoculum for subsequent survival and transmission experiments. Sub-samples of infected crowns (representing Day 0) were re-isolated for each experiment and 100% recovery of *M. phaseolina* were confirmed.

6.1. Survival I: Survival of *M. phaseolina* in buried crowns in a subtropical environment.

6.1.1 Aim

The aim of this experiment is to investigate the natural survival and longevity of *M. phaseolina* in infected strawberry crown debris buried in soil in subtropical conditions.

Location: Nambour, Queensland

Period: Oct 2017-Apr 2018, 6 months

List of treatments

- Whole crown
- Cut-half crown

6.1.2. Design

Inoculated whole crowns and crowns cut in half (longitudinally) were placed in plastic net bags (Figure 52) and buried 10 cm deep in a random block design (Figure 53). Whole crowns and half-crowns (with inner crown cortex exposed to the environmental elements) represent crown debris in the field when plants are incorporated back into the soil after the season. Ten samples each of whole crowns and cut-half crowns were collected at fortnightly intervals for 4 months, and monthly intervals thereafter for a total six-month period. Laboratory isolations, as described previously, were conducted to detect live *M. phaseolina*

Figure 52. Infected crown in a net bag ready for burial.

Figure 53. Buried infected crowns for study of pathogen survival in a subtropical environment

6.1.3. Results

Pathogen viability in crowns

M. phaseolina survived within buried strawberry crowns over the summer off-season in a sub-tropical production region. Although not significantly different ($P>0.05$), 30% of whole crowns and 20% of cut-half crowns tested harboured viable *M. phaseolina* for up to 24 weeks (Figure 52). The recovery of the pathogen from whole crowns was significantly different compared with the cut-half crowns at 6, 10 and 12 weeks ($P\leq0.05$).

A rapid reduction (70%) in percentage of cut-half crowns with viable *M. phaseolina* occurred over the first 4 weeks (Figure 54). Conversely, there was a meagre 30% decrease in the percentage of whole crowns with viable *M. phaseolina*. The sizeable decline in viable *M. phaseolina* may be due to the pathogen in cut-half crowns being exposed to the soil environment or competition from other microorganisms as the internal cortex tissue is colonized and decomposed. A similar rapid decline in the proportion of whole crowns with viable *M. phaseolina* occurred from 12 weeks.

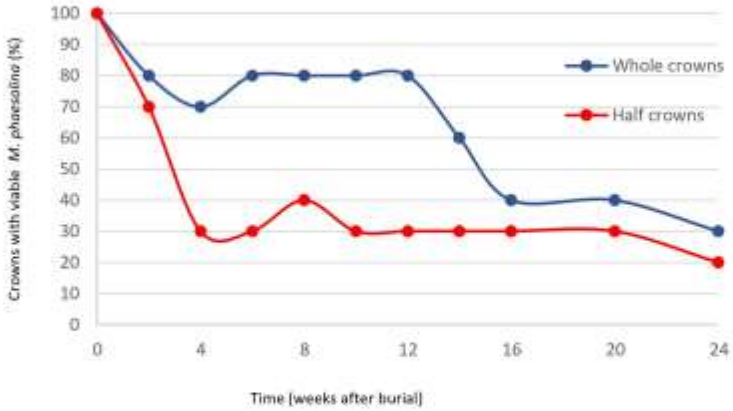

Figure 54. Percentage of infected crowns buried at 10 cm from which *M. phaseolina* was recovered over a 24-week period in sub-tropical conditions.

6.1.4. Conclusion

The experiment showed the detection of the pathogen declined over a 24-week period (Fig. 6). However, the pathogen was still detected in up to 30% of infected crowns, six months after burial in sub-tropical conditions. The results suggest the wide-spread industry practice of incorporating strawberry crop debris, including plants that are diseased, may play a part in increasing the inoculum levels in the soil.

6.2. Survival II: Survival of *M. phaseolina* in buried crowns in a temperate environment.

6.2.1. Aim

The aim of this experiment is to investigate the natural longevity of *M. phaseolina* in infected strawberry crown debris buried in soil in a temperate production environment, and to determine the impact of crown type (whole, cut-half) on the rate of decline in proportion of crowns with viable *M. phaseolina* throughout the winter off-season.

Location: Applethorpe, Queensland

Period: June 2019 – July 2020 (13 months)

List of treatments

- Whole crown
- Cut-half crown

6.2.2. Design

Inoculated crowns were buried at 10 cm deep in a randomised split block design (Figure 54). Twenty samples of each crown type were collected at fortnightly intervals for the first three months, then monthly through the summer and winter of the second year. Laboratory isolations were conducted to detect live *M. phaseolina*.

6.2.3. Results

Pathogen viability in crowns

M. phaseolina survived within buried strawberry crowns over-winter during the temperate production off-season. Approximately 15% of both whole and cut-half crowns (not significantly different), harboured viable *M. phaseolina* for up to 52 weeks (Figure 55). The rate of reduction in percentage of crowns with viable *M. phaseolina* was similar over the course of the year for both whole and cut-half crowns. In contrast to Survival I, there was no period of rapid reduction in percentage of crowns with viable *M. phaseolina*.

Figure 54. Infected buried crowns for a study of pathogen survival in a temperate environment.

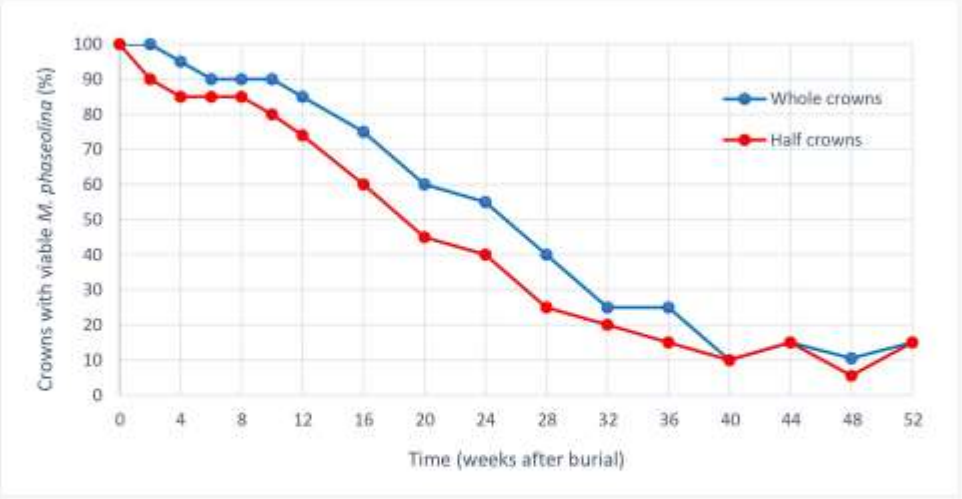

Figure 55. Percentage of infected crowns buried at 10 cm from which *M. phaseolina* was recovered over a 52-week period in temperate conditions.

6.2.4. Conclusion

In a temperate environment, *M. phaseolina* survives in buried strawberry crop debris for at least 12 months. The slow decrease in proportion of crowns with viable pathogen, and hence larger quantity of inoculum present for longer into the off-season, is likely related to the low soil moisture and low soil temperature during the winter. Severe drought conditions prevailed for the entire duration of the study. There was little decomposition of both crown types evident over the period of the study, potentially aiding the pathogen to survive within buried crowns. *M. phaseolina* can survive in buried strawberry crop debris to become a source of inoculum when a subsequent strawberry crop is planted. November-December is often when hot weather events, conducive to outbreaks of charcoal rot, start to occur in temperate strawberry production regions (e.g. Granite Belt). Yet the pathogen is still viable in around 30% of pieces of buried crowns from the previous crop, representing a large potential inoculum load in the soil. The wide-spread industry practice of returning strawberry crop debris to the soil is potentially perpetuating the soil inoculum load and exacerbating the difficulty of controlling the disease at sites with a history of charcoal rot.

7. Pot experiment 1: Disease transmission from infected crowns.

7.1. Aim

Building on from the survival experiments, the aim of this experiment was (a) to establish transmission of *M. phaseolina* from infected crowns and to new transplants, and (b) to determine the impact of crown type (whole or cut-half) and quantity on the rate of disease onset and plant death.

List of treatments

- 1 x whole crown
- 2 x whole crowns
- 1 x cut-half crown
- 2 x cut-half crowns
- 4 x cut-half crowns

7.2. Design

Whole or cut-half pieces of *M. phaseolina*-infected crowns, were buried in the potting mix when the bare-rooted, certified transplants of cv. Albion were potted to establish the experiment. Potted plants were grown

in a sterilised potting mix of 50:50 peat and sand, and maintained in the glasshouse. Each treatment consisted of 20 plants. Plants were monitored weekly, wilted plants recorded then dead plants collected for laboratory isolations to confirm presence of *M. phaseolina*.

7.3. Results

Disease symptoms were evident within four weeks of planting. At 12 weeks, plant mortality was highest for the greater inoculum load of 2 and 4 cut-half crowns (Figure 56). The plant mortality at 12 weeks in the equivalent inoculum load of whole crowns (ie 1 and 2 crowns) was significantly less (Table 18). Plant death was comprehensive in all treatments at six months.

7.4. Conclusion

Viable *M. phaseolina* in buried strawberry crop debris will infect new strawberry plants, leading to the development of charcoal rot. The rate of infection is faster where the surviving *M. phaseolina* is within cut pieces of strawberry crown, suggesting it is easier for the fungus to grow beyond the buried strawberry tissue to ultimately encounter a root of a strawberry plant. Infected strawberry debris is an important inoculum source for perpetuating charcoal rot in successive plantings. The annual removal of infected crop debris at the end of the season has the potential to reduce the inoculum load and risk of disease.

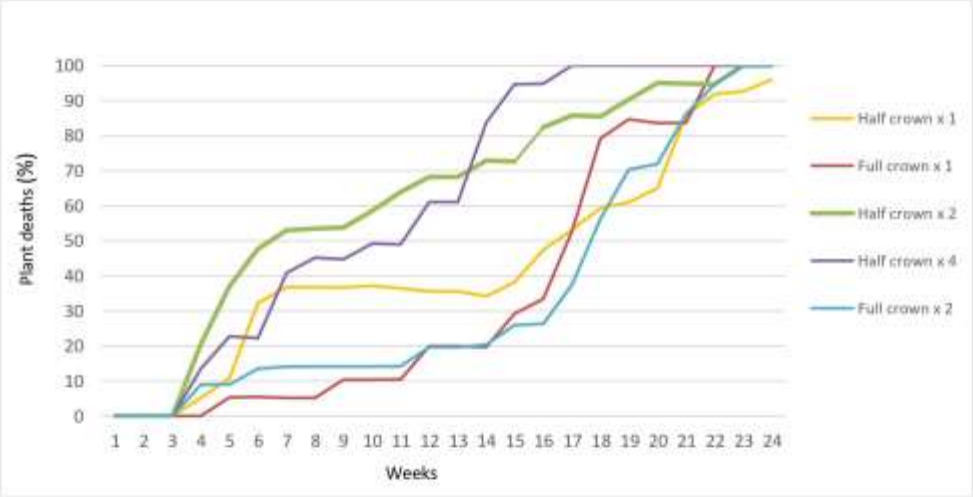

Figure 56. Percent mortality of cv. Albion plants over 24 weeks for different types (whole, cut-half) and quantity (half, one, two) of infected crowns. No plant deaths occurred in soils with no buried infected crowns (control).

Table 18. Plant deaths over 24 weeks of strawberry plants in pots with buried crowns infected with *M. phaseolina*. Letters indicate significant difference (p=0.05).

Treatment	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Half crown x 1	5	37 bc	36 ab	47 a	65 a	96
Full crown x 1	0	5 a	20 a	33 a	84 ab	100
Half crown x 2	21	54 c	68 c	82 b	95 b	100
Half crown x 4	13	45 c	61 bc	95 b	100 b	100
Full crown x 2	9	14 ab	20 a	26 a	72 ab	100

8. Alternative hosts of *M. phaseolina* in strawberry production systems

8.1. Aim

To identify alternative hosts of *M. phaseolina* amongst weeds, cover crop and non-strawberry crop plants growing in strawberry production systems.

8.2. Design

Up to five specimens of each plant species were collected from strawberry farms in the Sunshine Coast and Granite Belt regions in Queensland, and Yarra Valley, Victoria. Weeds were collected from within the strawberry plant bed and preferably sections along the row with dead plants, suggesting a high presence of charcoal rot. Also, weeds were collected toward the end of the season as they became more prevalent and the occurrence of charcoal rot was greatest. Necrotic and symptomless tissues from different plant parts (e.g. roots, crowns) were isolated on quarter-strength PDA plates and incubated for 7-10 days to determine presence of *M. phaseolina*.

8.3. Results

M. phaseolina was isolated from a sample of Blue Billy Goat Weed (*Ageratum houstonianum*) collected from a fruit farm in Elimbah, Queensland. A sample of white clover (*Trifolium repens*), and watermelon (*Citrullus lanatus*) growing as a rotation crop in from Victoria also returned positive for *M. phaseolina*. The pathogen was also isolated from buried debris of a sorghum crop (Figure 57) recently incorporated back into the soil. The pathogen was not recovered from any of the other plant species collected (Table 19).

8.4. Conclusion

Sorghum and watermelon are known hosts of *M. phaseolina* and their use as a cover crop and rotation crop plants in strawberry farms with a history of charcoal rot is ill-advised. Plant species that are not a host of *M. phaseolina* should be used instead of sorghum where cover cropping is part of the crop rotation strategy with strawberry production. *M. phaseolina* has a large number of hosts and the failure to recover the pathogen from the plants collected does not confer the weed species is not a host of *M. phaseolina*. Notwithstanding, weed species that are alternative hosts of *M. phaseolina* are a very minor source of inoculum due to the focus on maintaining a weed-free status throughout the growing season in modern strawberry production. Alternative hosts amongst cover crop and non-strawberry crop plants present a moderate risk of charcoal rot outbreaks and greater attention should be given to the choice of crops to grown in rotations on strawberry farms.

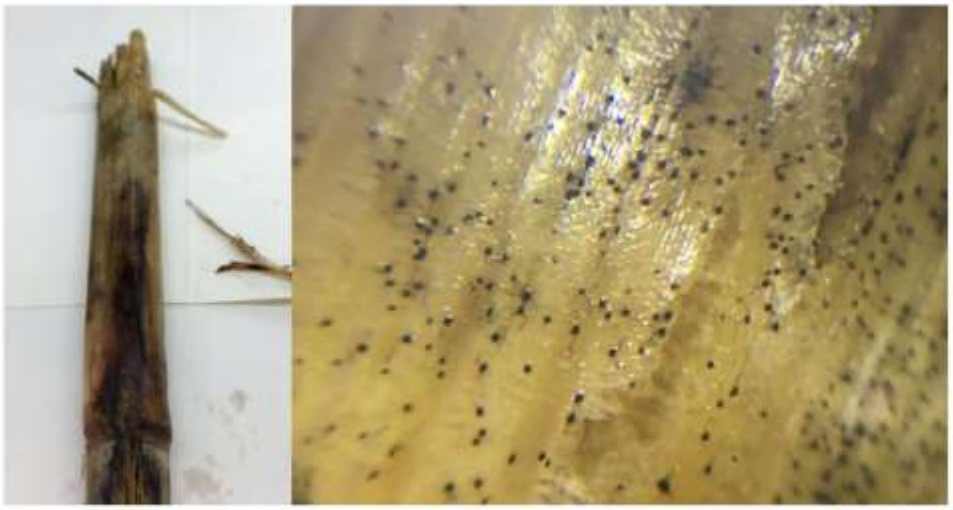

Figure 57. A piece of sorghum stubble (left) and a magnified section of the stubble showing microsclerotia of the pathogen (right).

Table 19. Plant species sampled for presence of *Macrophomina phaseolina* during the current study.

Common name	Botanical name
Barnyard grass	*Echinochloa crusgalli*
Blue billy goat	*Ageratum sp.*
Capsicum	*Cassicum annuum*
Celery	*Apium graveolens*
Chickweed	*Stellaria media*
Cobblers peg	*Bidens pilosa*
Corn	*Zea mays*
Couch	*Cynodon dactylon*
Cucumber	*Cucumis sativus*
Curled dock	*Rumex crispus*
Dandelion	*Taraxacum officinale*
Dead nettle	*Lamium amplexicaule*
Dill	*Anethum gravedens*
Fat-hen	*Chenopodium album*
Fig	*Ficus carica*
Flick weed	*Epilobium ciliatum*
Groundsel	*Senecio vulgaris*
Fleabane	*Conyza bonariensis*
Malva	*Malva* sp.
Marjoram	*Origanum majorana*
Marshmallow, small-flowered	*Malva parviflora*
Milkthistle, Sowthistle	*Sonchus oleraceus*
Nightshade, black berry	*Solanum nigrum*
Nutgrass	*Cyperus rotundus*
Pigweed	*Portulaca oleracea*
Potato	*Solanum tuberous*
Pumpkin	*Cucurbita moschata*
Red root amaranth	*Amaranthus retroflexus*
Shepherd's purse	*Capsella bursa-pastoria*
Silverbeet	*Beta vulgaris*
Sorghum (cover crop)	*Sorghum bicolor*
Sow thistle	*Sochus oleraceus*
Spear thistle	*Cirsium vulgare*
Sunflower	*Helianthus annus*
Thai basil	*Ocimum basilam*
Tomato	*Solanum lycoparsiem*
Watermelon	*Citrillus lanatus*
White clover	*Trifolium repens*
Winter grass	*Roa annua*
Wireweed	*Polygonum aviculae*
Vietnamese mint	*Persecaria* sp.
Zucchini	*Cucrbita pepo*

9. Field study: Integrated management system

9.1. Aim

Determine the impact of removal of strawberry crop debris on the level of charcoal rot in the subsequent crop.

To determine the effectiveness of strawberry crop debris removal in combination of the best available fumigant, application method, and TIF, in controlling charcoal rot.

Location: Stanthorpe, Queensland

Period: May 2018 – May 2020.

Treatments:

- Crop debris removed, Tri-Form® 80, under TIF.
- Crop debris incorporated, Tri-Form® 80, under TIF
- Crop debris removed, Tri-Form® 80, under TIF, plus metham sodium
- Crop debris incorporated, Tri-Form® 80, under TIF, plus metham sodium

9.2. Design

In May 2018, a field trial was established at the end of the fruit harvest season on a strawberry farm in the Granite Belt with a history of charcoal rot. Old strawberry plants were removed from 15 m sections along rows (Figure 58), the paddock was cultivated and remained fallow over the 2018-19 summer. The site was fumigated (June 2019) planted with certified cv Monterey transplants. Fruit harvest commenced early October and continued through to May 2020. All plants (32) in a 5 m plot within the treated section (15 m) were harvested up to 3 times a week. Berries were graded into marketable quality or reject, as per seasonal industry standards, with the number of berries and total fruit weight recorded. Plant deaths across the 15 section of row was recorded several times throughout the growing season.

Figure 58. Strawberry crop debris removed from plots at the integrated systems field trial.

9.3. Results

There was no significant difference in cumulative total fruit yield at the end of the season between the crop debris removed and crop debris incorporated treatments (Figure 59). No significant difference in plant deaths was recorded across treatments.

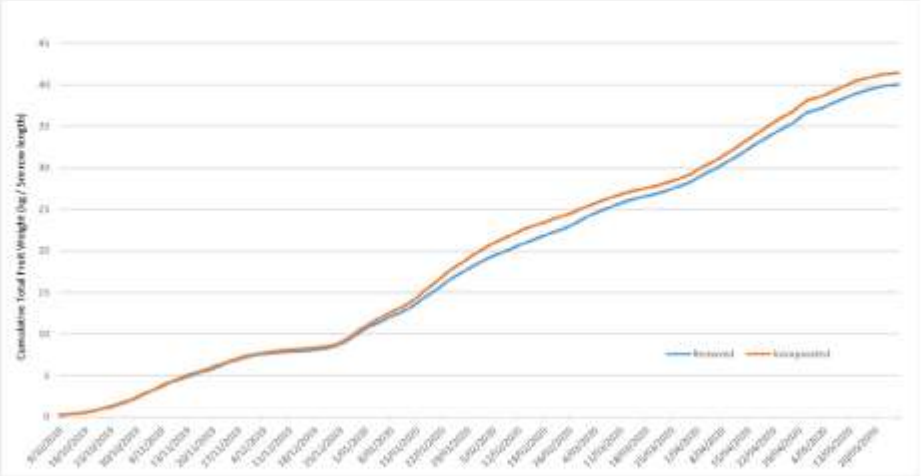

Figure 59. Cumulative total fruit weight in season 2019-20 of cv. Monterey, at a *M. phaseolina* infested site where crop debris was removed or incorporated at the end of the previous season (2018).

9.4. Conclusion

Whilst the survival and transmission studies established the principle that infected strawberry crop debris is be a major inoculum source for outbreaks of charcoal rot in the subsequent strawberry crop, removing crop debris did not lead to a significant improvement in fruit yield or reduced plant deaths after one growing season. This could be due to the impact on reducing the soil inoculum by removing infected crop debris being masked by either a very high background inoculum level or the efficacy of the fumigants used. It may take several seasons (2-5 crops) of repeated removal of plant debris for a cumulative impact on reducing pathogen inoculum in the soil to have a visible effect on disease.

10. General discussion, outcomes & conclusions

Growers can adopt the following techniques to improve control of charcoal rot:

- Research in this project showed that fumigation with formulations of 1,3-dichloropropene and chloropicrin (e.g. Tri-Form® 80 and Agrocelhone FE) and/or EDN have the capacity to reduce charcoal rot.
- Use totally impermeable films (TIFs) instead of standard films made of low-density polyethylene to seal soils when fumigating.
- Shank injection proved an effective method of application for these fumigants.
- Use broad-acre fumigation (treating the whole paddock) instead of standard strip/bed fumigation.
- Only apply soil fumigants under the correct environmental conditions of soil moisture, temperature, tilth, organic matter content (see product labels).
- Terminate old strawberry crops with metham sodium (400 L/ha) at the end of the growing season.
- Implement good farm hygiene practices that limit the movement of soil within and between strawberry farms when conducting farm operations.
- Remove infected crowns when possible and avoid incorporating back in the ground to reduce risk of pathogen population increasing.
- Avoid planting sorghum as cover-crop and alternative hosts of *M. phaseolina* (e.g. watermelon) in strawberry cultivation blocks with a history of charcoal rot.