Maintaining biosecurity standards for soil-borne pathogens and weeds in the strawberry runner industry

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Project BS07014

Mattner S.W. *et al.*

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Final report for Horticulture Australia Ltd Project BS07014 (July 2002)

Maintaining biosecurity standards for soilborne pathogens and weeds in the strawberry runner industry

The Australian strawberry industry continues to face one of its greatest challenges of the modern era – the phase-out of methyl bromide (MB) due to its ozone depleting properties. For 60 years, industries have used MB to disinfest soils of pathogens, weeds and pests, and to maximise yields. This project was conducted to identify alternatives for those industries applying to the UN for critical-use exemptions to retain MB use - specifically strawberry runners and fruit. Through this research, the strawberry fruit industry has been able to phase out methyl bromide completely, however, the runner industry is still seeking suitable alternatives. The project has enabled reductions in MB use by 6 tonnes and allowed Australia to meet its obligations under the Montreal Protocol. The identification of alternatives to MB through this and previous projects has prevented losses in the Australian horticultural industries of around $100 million annually.

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Contents

1. Media Summary

Methyl bromide (MB) is a highly effective soil fumigant that is being phased-out due to its ability to degrade the ozone layer. The strawberry runner industry is the last remaining user of MB for soil fumigation in Australia. Phase out of MB in the industry was reliant on trials in this project and registration of methyl iodide (MI), however its withdrawal from Australia means other alternatives will need to be developed. Australia is only one of three remaining countries applying for 'Critical Use Exemptions' (CUEs) under the Montreal Protocol to retain the use of MB. Many other countries have been able to adopt fumigant alternatives but these have proven ineffective in the heavy soils and climate experienced in the southern Australian production districts at Toolangi, where strawberry runners are grown. In spite of this, the project has demonstrated many other successful outcomes.

- MB use in the Australian strawberry runner industry has reduced by 6 tonnes since the start of this project owing to identification of alternatives and strawberry varieties (lower chill nurseries) for specific growing regions in northern Australia that could adequately transition to existing alternatives.
- Data showed that soil fumigation with the alternative MI provides the same biosecurity and certification standards in terms of pathogen control, weed control, runner yields and quality, and human safety as MB.
- Data on MI was submitted to the Australian Pesticides and Veterinary Medicines Authority (APVMA), Office of the Chief Plant Protection Officer, and Strawberry Certification authorities to expedite approvals for registration, market access and use in certification schemes. Since completing this work, however, the registrant has notified the APVMA in June 2012 that it has withdrawn the application for MI due to the current high cost of iodine worldwide.
- Data from this project supported the registration of two new alternatives (PicPlus® and Telone C60®). Although these alternatives proved unsuitable for strawberry runners, they were effective for fruit growing and are now the two most widely used fumigants in the strawberry fruit industry.
- New application techniques, such as drip fumigation, were identified that improve the efficacy of alternatives in the strawberry fruit industry. Data was used to support the registration of this technique for some MB alternatives (e.g. Telone® products).
- New alternative fumigants or methods were shown to be effective in trials and show promise for replacing MB in the runner industry with further development, e.g. recaptured MB from quarantine applications combined with chloropicrin (Pic).
- The project also identified new fungicide spray regimes that combine different chemical classes for managing *Gnomoniopsis fructicola* in runners and this will reduce the likelihood of fungicidal resistance.

Without registration of MI, there are few alternatives for the runner industry to phase-out MB. Industry and government need to consider: (1) ongoing CUE applications for MB, or (2) accepting the risks of reduced biosecurity levels in runners with current MB alternatives and determining the legal responsibility for this risk.

2. Technical Summary

Methyl bromide (MB) is an effective soil fumigant that was widely used in Australian horticulture, but is being phased-out due to its ability to degrade the ozone layer. This project was conducted to find alternatives to MB for the strawberry runner industry. The issue for the industry is producing certified transplants with MB alternatives without compromising biosecurity and market access standards. The runner industry has reduced its use of MB by 6 tonnes since the start of this project. This happened by identifying alternatives for specific growing regions and strawberry varieties (lower chill nurseries) that could adequately transition to existing alternatives.

In regions still using MB, data showed that soil disinfestation with the alternative methyl iodide (MI) / chloropicrin (Pic) provides the same biosecurity and certification standards in terms of pathogen control, weed control, and runner yields and quality as MB/Pic. Air monitoring and dispersion modelling showed that emissions of MI/Pic from treated soil were extremely low risk to bystander and environmental safety. Only 6% of applied MI was emitted to the atmosphere over a 10 day period. The concentrations measured offsite were much lower than the U.S. Environmental Protection Agency (U.S. EPA) reference concentration and dispersion modelling (PERFUM2) predicted zero buffer zones for MI:Pic would be required in runner growing districts in Australia.

Data on MI was submitted for approvals for registration, market access and use in certification schemes. However, since completing this work, the chemical company seeking registration (Arysta Life Science) of has withdrawn the application for registration of MI (June 2012) due to the current high cost of iodine worldwide. Data from this project assisted in the registration of two new alternatives (PicPlus® and Telone C60®). These products proved unsuitable for runners, but they were effective for fruit growing and are now the two most widely used fumigants in the strawberry fruit industry. New application techniques, such as drip fumigation, were identified that improve the efficacy of some alternatives in the strawberry fruit industry. Data was used to support the registration of this technique for some alternatives (e.g. Telone® products).

New alternative fumigants or methods were identified for replacing MB in the runner industry. For example, two completed trials in the runner industry showed that recaptured MB from quarantine applications, co-applied with Pic, provided equivalent pathogen and weed control and runner yields to traditional formulations of MB. The recapture and reuse of MB from quarantine applications is currently exempt from phase-out under the terms of the Montreal Protocol. This is the first time this alternative has been identified worldwide, but requires further experimentation to develop the product for registration.

The project identified new fungicide spray regimes that combine different chemical classes for managing *Gnomoniopsis fructicola* in runners and this will reduce the likelihood of fungicidal resistance. Previously the industry had relied on fortnightly sprays with a single fungicide, Prochloraz, for the last 20 years and now has other options.

To achieve the project outcomes a variety of technology transfer tools were employed. Over its duration (5 years), this project delivered: 21 grower newsletter articles and manuals, 19 oral presentation days to industry, 17 technical reports to government agencies, 27 scientific conference papers, 1 university thesis, 7 refereed scientific papers and 2 book chapters.

Without registration of MI, there are few one-off alternatives for the runner industry to phase-out MB. Industry and government need to consider: (1) ongoing critical use exemptions for MB, or (2) accepting the risks of reduced biosecurity levels in runners with current MB alternatives and determining the legal responsibility for this risk. Further research should be

directed towards developing integrated disinfestation systems that combine existing fumigant, fungicide, nematicide, herbicide and biofumigant chemistries for control of soil-borne pests in the runner industry.

3. Introduction

3.1 The global phase-out of methyl bromide for soil disinfestation

Soil disinfestation is the process of reducing or controlling pathogens, weeds, nematodes and pests in soil prior to planting crops. For 50 years, mixtures of methyl bromide (MB) and chloropicrin (Pic) have been the most widely used and effective chemical fumigants for soil disinfestation in the world. However, the bromine from MB is 60 times more efficient at destroying ozone than the chlorine from the well known ozone-depleters, chlorofluorocarbons (CFCs). For this reason, MB was added to the *Montreal Protocol on Substances that Deplete the Ozone Layer* and was scheduled for phase-out for soil disinfestation purposes in developed countries, including Australia, by 2005.

3.2 Impact of MB phase-out on the strawberry industry

The MB phase-out threatened the domestic biosecurity of many Australian horticultural industries, including the strawberry industry. The industry was challenged with finding an alternative that provided the same level of pathogen and weed control as MB:Pic mixtures, in order to maintain the high health status and yields of strawberry transplant and fruit production. In 1995, the strawberry industry used 142 tonnes of MB p.a. for soil disinfestation – 20% of the national use (Methyl Bromide Consultative Group, 1998). Without a suitable replacement the strawberry industry stood to lose c. 35% in yields, equivalent to $84 million p.a.

3.3 Strawberry multiplication and the role of MB

Strawberry transplants (runners) produced in Victoria (80% of national production) go through four generations of multiplication. The multiplication process follows a set of conditions (the Victorian Runner Certification Scheme, VRCS) that governs the production and certification of runners – from the importation of new varieties to the distribution of runners to Australian strawberry fruit growers. Strawberry runner certification schemes in other states (i.e. Queensland and Tasmania) follow a similar multiplication system as Victoria. The VRCS is overseen by the Victorian Strawberry Industry Certification Authority (VSICA). Among the aims of the VRSC is to ensure that runners are certified as disease tested and to maintain domestic biosecurity in the strawberry industry. Plants are monitored and tested for diseases through all stages of the multiplication process:

Generation 1: Nucleus stock plants are grown and tested for the presence of viral, bacterial and fungal pathogens by the Victorian Department of Primary Industries, under the supervision of VSICA.
Generation 2: Foundation stock* are produced in aphid-proof screen houses within the Toolangi Plant Protection District. Plants are constantly monitored for diseases by the Manager of VSICA, which includes pathogen testing.
Generation 3: Mother stock* are produced by select members of the Toolangi Certified Strawberry Runner Growers Co-operative (TCSRGC) in open-fields within the Toolangi Plant Protection District. Runners are regularly inspected by the Manager of VSICA, which includes pathogen testing as necessary.
Generation 4: Certified stock* are produced by members of the TCSRGC in open fields within the Toolangi Plant Protection District. Runners are regularly inspected by the Manager of VSICA for diseases, which includes pathogen testing as necessary.

* At the start of this project (2008), areas for production of foundation, mother and certified stock were fumigated as specified in the VRCS by a 50:50 mixture of MB:Pic at 500kg/ha. In 2012, VSICA took over the production of nucleus and foundation stock, and now uses soil-less substrates without MB:Pic fumigation for these generations only.

Soil disinfestation is the key strategy used by runner growers to safeguard against the build up of soil-borne diseases and weeds through three stages of the multiplication process. Currently, MB:Pic mixtures are the only approved fumigants for this purpose. In 2008, the Australian strawberry runner industry used 35.75 tonnes of MB for soil disinfestation. The rules of the VRSC state:

'3.1.6: The site must be fumigated with a registered chemical by a method approved by VSICA. Currently, VSICA approves soil fumigation with a 50:50 mixture of methyl bromide and chloropicrin applied at a rate of 500 kg/ha under a plastic film'

Strawberry runners produced under the VRCS are delivered as bare-rooted transplants to strawberry fruit growers in every state of Australia. Market access of runners into some states (e.g. Western Australia, Tasmania) is supported by them being produced in MB-treated soils (Mattner et al., 2010). The Victorian runner industry currently produces 50 million transplants valued at c. $15 million p.a. The Australian strawberry fruit industry was recently valued at $240 million annually (PISC, 2009).

3.4 Registered chemical alternatives to MB for soil disinfestation

From 1995, a National MB Research and Communication Program was initiated that aimed to identify alternative soil disinfestation systems to MB for horticultural industries. The program conducted more than 100 trials nationally investigating more than 20 fumigant alternatives and 40 non-fumigant alternatives for strawberry (Mattner et al. 2008). The development and commercialisation of some of these alternatives allowed most Australian horticultural growers to cease use of MB for soil disinfestation by 2005-06, without significant increases in pest and disease pressure on-farm or loss in profits. The program resulted in the registration of three chemical actives in Australia for soil disinfestation: 1,3 dichloropropene (Telone® II, Telone® C-35), methyl isothiocyanate (Basamid®, Metham Sodium and Envirofume®), and chloropicrin (Chlorofume®). In strawberry fruit trials, these alternatives consistently provided effective alternatives to MB for soil disinfestation (Mattner et al., 2002; 2005; 2008). However, in trials in the strawberry runner industry, they sometimes caused crop failure and poor disease and weed control (Mattner et al. 2005; 2008). For this reason, VSICA does not approve their use as alternatives to MB for soil disinfestation in the strawberry multiplication scheme.

The difficulties in the runner industry with using the registered alternative fumigants lie with their significantly higher high boiling points and lower vapour pressures compared with MB. This combined with the cold temperatures that are required to grow runners (i.e. soil temperatures below 5ºC during the fumigation season) and the heavy soils (clay – clay/loams) with high organic matter (c. 5%) within the Toolangi Plant Protection District mean that the registered alternative fumigants fail to adequately enter the gaseous phase which is essential for effective soil fumigation. These factors (i.e. cold temperatures and heavy soils with high organic matter) led to residues of the alternative fumigants persisting for long periods in soil, causing significant phytotoxicity issues in the strawberry runner crop. For example, soil disinfestation with 1,3-dichloropropene (1,3-D):Pic mixtures resulted in yield losses in strawberry runner

crops of up to 40%, even after plant-back periods of 3 months (Mattner et al. 2005; 2008). Furthermore, soil disinfestation with alternative fumigants led to unacceptable weed control (e.g. weed emergence was 3-fold greater in plots treated with Pic than in those treated with MB) and pathogen control (e.g. soil fumigation with 1,3-D:Pic mixtures resulted in an increase in the incidence of crown rot, caused by *Phytophthora cactorum*, in strawberry runners) (Mattner et al., 2005; 2008).

3.5 Critical-use exemptions for MB in the strawberry runner industry

Under the terms of the *Montreal Protocol*, industries that can demonstrate they have no technically or economically feasible alternatives to MB can apply annually to the United Nations (UN) for a critical-use-exemption (CUE) to retain its use. Due to the reduced efficacy and difficulties in adopting registered alternative fumigants, the strawberry runner industry has applied annually for CUEs since 2005. Currently, the industry holds a CUE until 2014, but it is unlikely that the UN will grant them for long periods into the future. At the start of this project in 2008, the Australian runner industry held a CUE for 35.75 tonnes of MB p.a., and there was urgent need to identify alternatives to safeguard domestic biosecurity standards and market access in the strawberry industry.

3.6 Project Aims

This project conducted research over a 5-year period to identify and support the registration of new alternatives to MB for soil disinfestation in the strawberry runner industry that: (1) maintained biosecurity, certification and market access standards, and (2) were safe for operators, bystanders, and the environment.

4. Commercial Trials of Methyl Iodide (Activity 1)

4.1 Introduction

Early research with methyl iodide (MI) established it as a one-to-one replacement for MB for soil disinfestation (Becker et al., 1998, Hutchinson et al., 1999, 2000, Zhang et al., 1998). This is because MB and MI have similar physical and chemical properties (Table 3.1), and activity against pests. For example, the mode of action of MB and MI against pathogens and weeds is through bimolecular nucleophilic displacement (S_N2) reactions with amino acids and peptides in the target organisms. MI reacts faster than MB under the majority of S_N2 reactions that have been studied (Ohr et al., 1996), and therefore MI has greater potential for controlling pathogens and weeds than MB. Unlike MB, however, MI rapidly breaks down when exposed to light through phytolysis. This gives MI a low atmospheric residence time, and a correspondingly low stratospheric ozone-depleting potential (0.016 compared with 0.65 for MB) (Zhang et al., 1998).

In 2002, Arysta LifeSciences imported experimental quantities of MI into Australia for trialling through the National MB Research and Communication Program. Small-scale trials with mixtures of MI:Pic (30:70 and 50:50 at 300-500 kg/ha) consistently showed equivalent efficacy to MB:Pic (50:50 at 500 kg/ha) for controlling soil-borne pathogens and weeds, and for producing runner yields (Mann et al., 2005; 2008; Mattner et al., 2005; 2008). Additionally, MI has a significantly lower boiling point and higher vapour pressure than other alternative fumigants (Table 3.1), thereby minimising the risks of fumigant-induced phytotoxicity in runner crops, and inconsistent pathogen and weed control experienced with other registered alternatives. The results from this research gave the runner industry the confidence to cite MI as their best opportunity for phasing-out MB in their annual applications for CUEs to the UN. Despite this, certification agencies requested commercial trials demonstrating the efficacy of MI over a larger scale before they would approve its use within runner multiplication schemes. This was seen as crucial, since phytotoxicity issues with other alternative fumigants (e.g. 1,3-D) only appeared in commercial scale trials, rather than in small-plot trials where conditions were more controlled. Arysta LifeSciences lodged a registration application for MI with the Australian Pesticides and Veterinary Medicines Authority (APVMA) in 2005, and further commercial trials were important in supporting this application. At the time of writing this report, Arysta had just notified the APVMA that registration will not longer be sought in Australia.

The aim of the trials described in the chapter was to evaluate efficacy of MI:Pic mixtures, compared with MB:Pic, for pathogen and weed control, and runner yields in commercial-scale applications.

4.2 Materials and Methods

Fourteen commercial trials investigating mixtures of MI:Pic for soil disinfestation in the strawberry runner industry were established on commercial strawberry runner farms in the Toolangi Plant Protection District between 2008 and 2011. Trial beds were prepared and maintained for strawberry runner production using standard industry practices. Trials were conducted on flat rows, which were broad-acre fumigated following normal soil preparation (rotary hoeing and incorporation of lime). Individual plots were between 50 - 500 m in length and 10 – 60 m in width. All fumigants were shank injected into soil to a depth of 20 cm through tynes spaced 20 cm apart using a commercial rig (R&R Fumigation Services), and the soil surface sealed with low density polyethylene (30 µm thickness). Fumigation occurred between March to June each year. Between 1-2 weeks after fumigation, barrier film was removed and the soil allowed to air prior to planting. Plots were planted with strawberry mother plants 10-12 weeks after fumigation (August to September) spaced 50 to 70 cm apart. Strawberry runners were harvested seven to ten months after planting (from March to June), depending on the variety grown. In general, untreated controls could not be included in trials because they were on commercial farms.

Except where otherwise stated, data were analysed using ANOVA as performed on the GENSTAT v. 12 statistical package (Lawes Agricultural Trust, IACR Rothamsted). Homogeneity of variance was determined by examining plots of fitted values versus residuals, while histograms of residuals were used to assess normality of distribution. Data transformations were made where appropriate. Fischer's LSD test was used to identify significant differences ($p \leq 0.05$) between treatment means.

4.3 Results

4.3.1 Crop growth and yields in commercial trials

Commercial trials consistently demonstrated the potential for MI:Pic to deliver equivalent strawberry runner yields to MB:Pic.

In 2008-09, large blocks on seven commercial runner farms in the Toolangi Plant Production District were fumigated with MI:Pic (30:70) at 500 kg/ha, and MB:Pic (50:50) at 500 kg/ha (the standard rate of MB:Pic) and 375 kg/ha. Each farm represented one replicate of each treatment, and treated soils were all planted with the strawberry variety 'Albion'. The establishment of mother plants, as measured by stolon numbers and lengths at two months after planting, was equivalent in the standard rate MB:Pic and MI:Pic treatments, but significantly less in the low rate MB:Pic treatment (Table 4.1). By final harvest, average commercial yields were statistically equivalent between treatments (Figure 4.1).

Table 4.1. Establishment of strawberry mother plants (2 months after planting, var Albion) in soils treated with various fumigants in commercial trials in Toolangi, Victoria in 2008-09.

Treatment	Stolon Number per Mother Plant	Leaf Number per Mother Plant	Length of the Primary Stolon (mm)
MB:Pic (50:50) 500 kg/ha	1.78 b	5.87	22.85 b
MI:Pic (30:70) 500 kg/ha	1.71 b	5.65	22.13 b
MB:Pic (50:50) 375 kg/ha	1.03 a	5.95	12.85 a
LSD (p = 0.05)	0.62	0.55	7.93

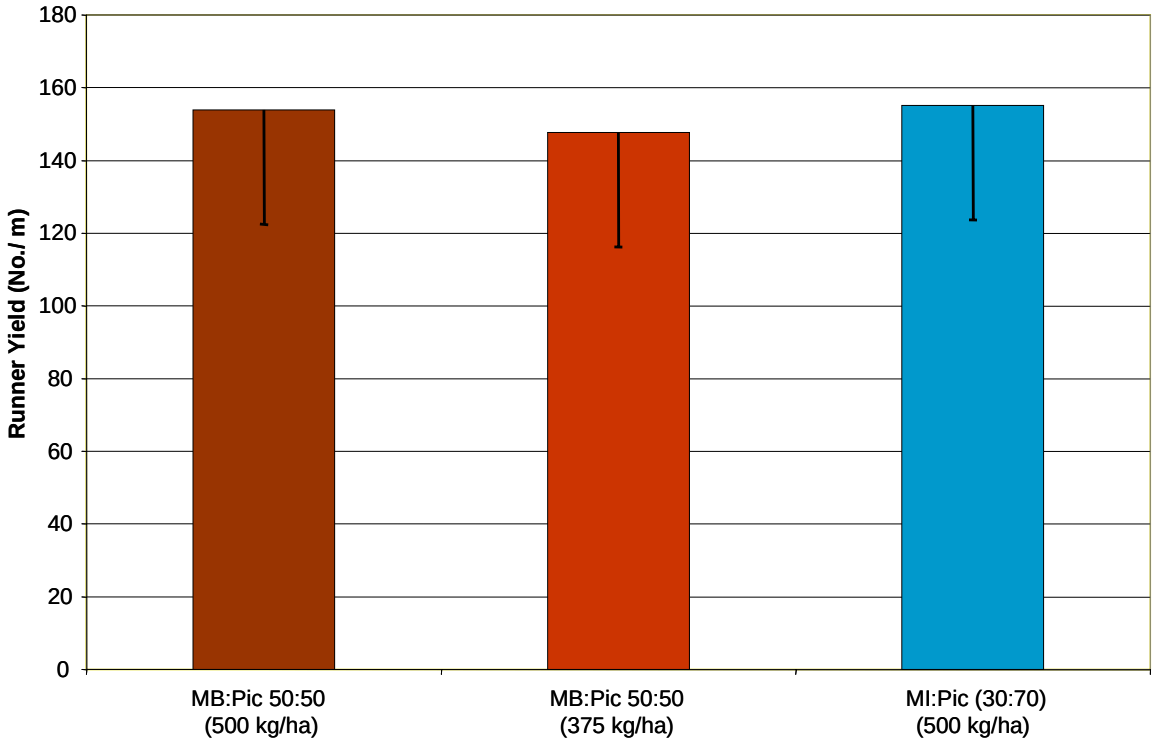


Figure 4.1. Yields of strawberry runners (var. Albion) grown in soils treated with various fumigants in commercial trials at Toolangi, Victoria in 2008-09. Bars are the least significant difference where p = 0.05.

In 2009-10 and 2010-11, three large-scale trials were conducted each year. Trials were conducted as completely randomised block designs with three to four replicates of each treatment. Treatments consisted of MI:Pic (50:50) at 350 kg/ha (the newly proposed label rate for MI formulations), and MB:Pic (50:50) at 500 kg/ha and 350 or 400 kg/ha. Strawberry varieties investigated in trials included Gaviota, Festival, Albion, Aromas and San Andreas. There were no significant differences in the establishment of mother plants (e.g. plant height, Figure 4.2) or commercial runner yields (Figures 4.3 and 4.4) between fumigant treatments in any of the trials. However, yields in fumigated plots were significantly greater than those in untreated plots (Figure 4.4).

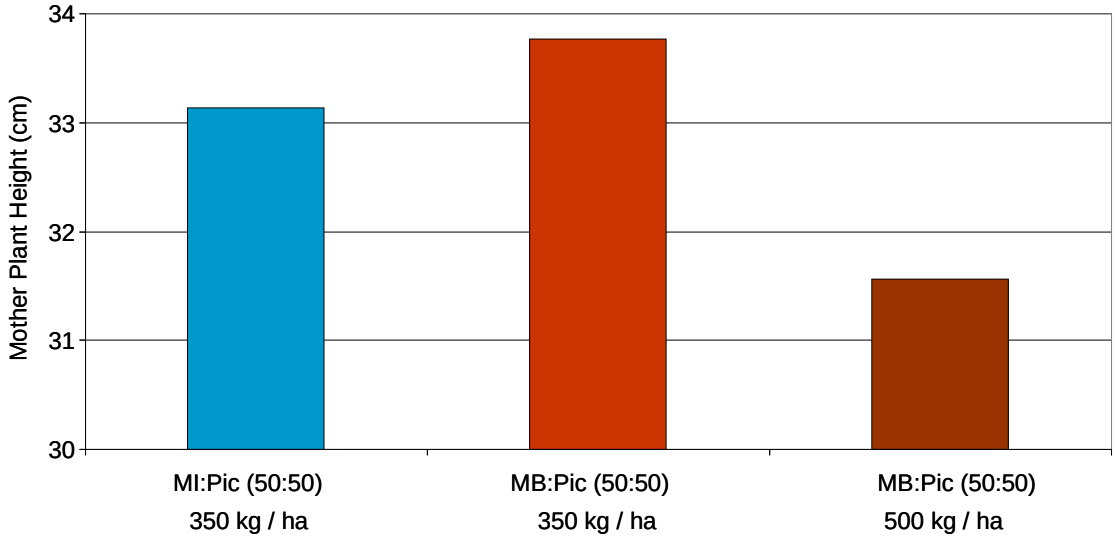


Figure 4.2. Height (cm) of mother plants (2 months after planting, var. Festival) grown in soils treated with various fumigants in a strawberry runner trial in Toolangi, Victoria in 2009-10. There was no significant difference between treatments, where $p \leq 0.05$.

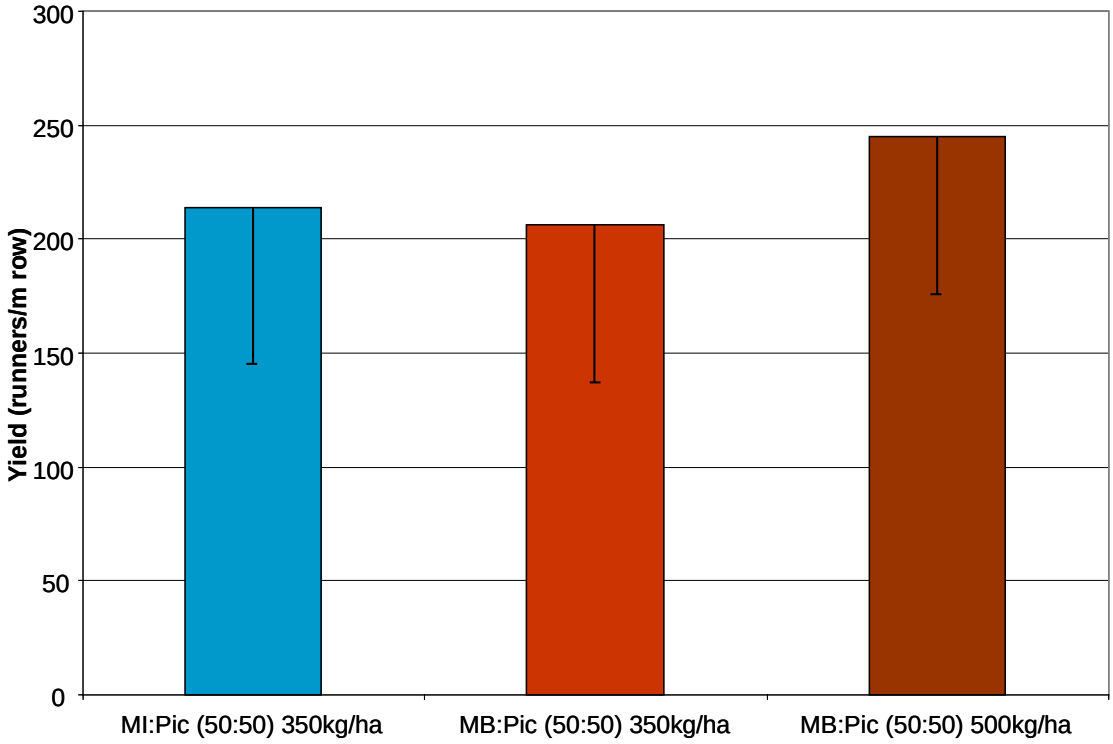


Figure 4.3. Yields of strawberry runners (var. Festival) grown in soils treated with various fumigants in a commercial trial at Toolangi, Victoria in 2009-10. Bars are the least significant difference where $p = 0.05$.

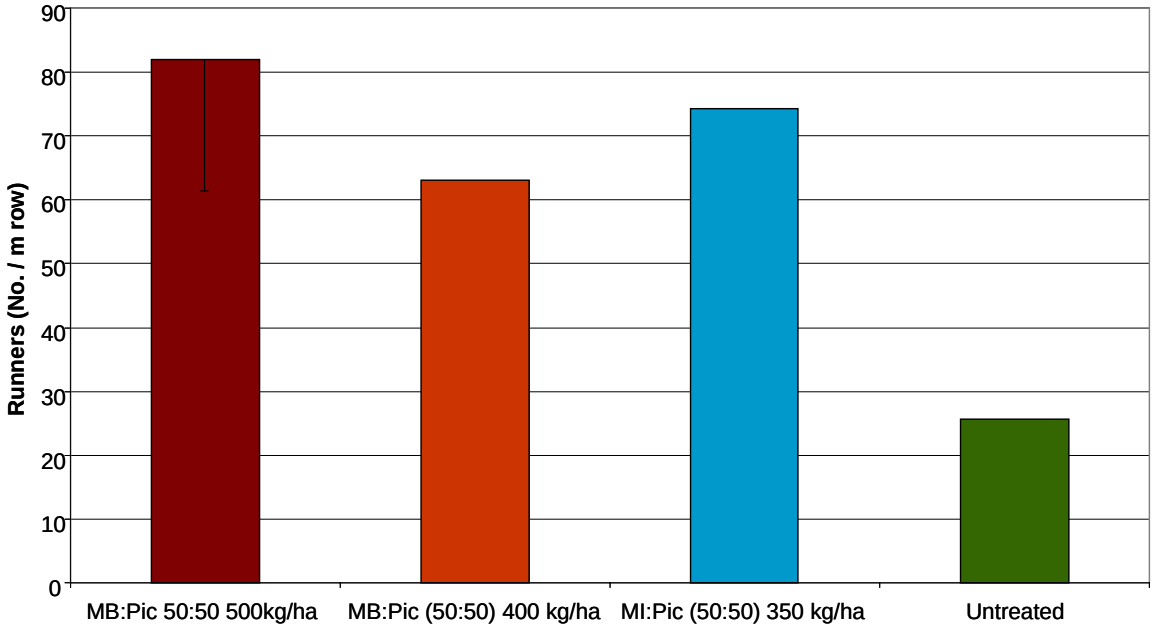


Figure 4.4. Yields of strawberry runners (var. Gaviota) grown in soils treated with various fumigants in a commercial trial at Toolangi, Victoria in 2010-11. The bar is the least significant difference where p = 0.05.

There was no evidence of fumigant-induced phytotoxicity in mother plants in any treatment in any year of the commercial trials.

In 2010-11, a separate trial investigating the spatial variability of crop yields in MI:Pic treated soils was conducted. Here, a 60 × 60 m block within an 80 × 80 m paddock in the Toolangi Plant Protection District was fumigated with MI:Pic (50:50) at 500 kg/ha. A ryecorn cover crop was sown (60 kg/ha) in the paddock four weeks after fumigation, as an indicator of plant growth. At harvest (three months after sowing), 32 biomass samples were taken from set areas inside and outside the treated block. In this procedure, ryecorn plants in a 1 m quadrat were dug out, roots washed free of soil, and all plant dried (80ºC for 4 days) and weighed. Analysis by Student's t-test showed that crop biomass in the treated area (480±35 g/m) was significantly higher (120%) than in the non-treated area (220±16 g/m). Spatial variation (Spatial Analysis as performed on Genstat v. 12) showed that biomass within the treated area was uniform, but fell markedly immediately outside of this area (Figure 4.5).

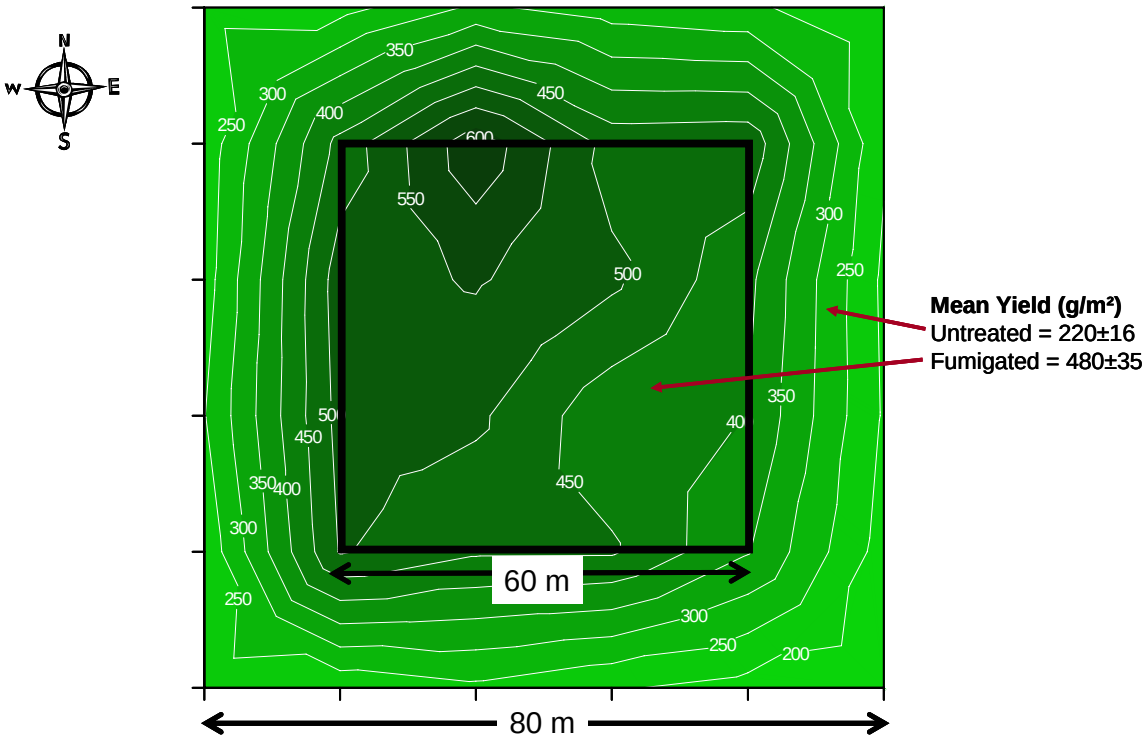


Figure 4.5. Spatial variation of ryecorn yield (g/m²) within a block partially fumigated with MI:Pic (50:50) at 500 kg/ha in Toolangi , Victoria in 2010-11.

A trial established by Mattner et al. (2008), investigating the long-term effects of soil disinfestation with MI:Pic and other fumigants was continued in the current project. The trial was conducted as a randomised complete block design with three replicates. In the trial, the same plots were treated annually for five consecutive years (from 2006-07 to 2010-11) with MI:Pic (50:50) at 350 kg/ha, 1,3-D:Pic (65:35) at 500 kg/ha, MB:Pic (50:50) at 500 kg/ha and an untreated control, and used to grow strawberry runners (var. Gaviota). Results showed that yields in MI:Pic-treated plots were consistently equivalent to those in MB:Pic-treated soils, and significantly greater (by an average of 60%) than in the untreated control (Figure 4.6). By comparison, yields in plots treated with 1,3-D:Pic were significantly below those in the MB:Pic treatment in three of the five years. In these years, mother plants grown in 1,3D:Pic-treated soils showed symptoms consistent with fumigant-induced phytotoxicity (Greer et al., 2005).

4.3.2 Pathogen control in soil

Commercial trials consistently demonstrated the ability of MI:Pic to reduce inoculum of pathogens in soil to the same level as MB:Pic.

In the commercial trials conducted in 2008-09 (see Section 4.3), a motorised auger was used to take 10 random soil samples (W sampling pattern) per plot at depths of 10 cm, 30 cm and 80 cm, 3 months after sowing. DNA was extracted from homogenised soil samples and quantitative PCR performed for the pathogens *Rhizoctonia solani* AG 3 and *Pythium* spp. (clade F) using propriety techniques by the South Australian Research and Development Institute. *Pythium* clade F contains many of the *Pythium* species that

are pathogenic to strawberries (Levesque and de Cock 2004). *Rhizoctonia solani* AG6 and

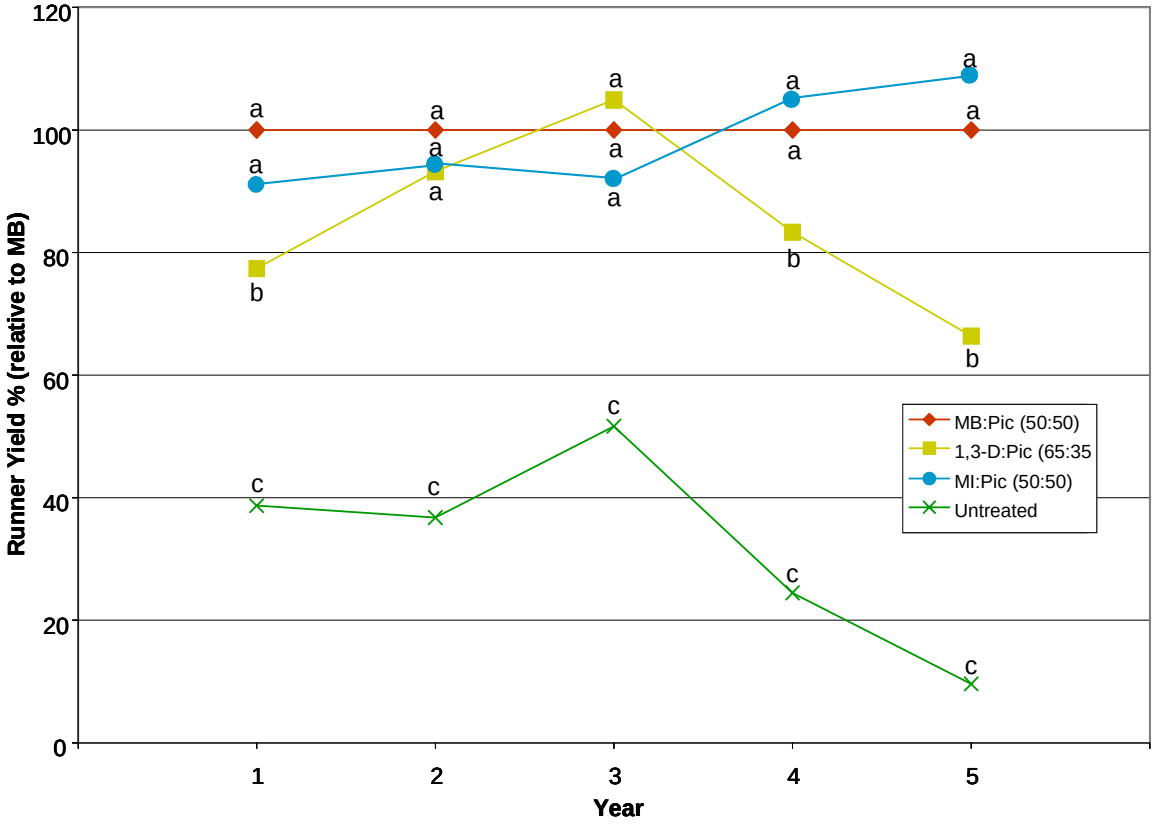


Figure 4.6. Yields of strawberry runners (var. Gaviota) grown in soils treated with various fumigants over five consecutive years at Toolangi Victoria. Data points followed by different letters in each year are significantly different, where $p \leq 0.05$.

AG2.1 are pathogenic on strawberry, but AG3 was the most prevalent in this soil, so was used as a surrogate model. These fungal species were selected because pre-fumigation tests showed they were present in field soils, whereas other strawberry pathogens (i.e. *Verticillium dahliae* and *Meloidogyne* spp.) were not. Results showed that MI:Pic (30:70) at 500 kg/ha reduced DNA levels of *R. solani* in soil by 99-100% and *Pythium* spp. by 85-97% (Table 4.2). This effect was statistically equivalent to the standard MB:Pic treatment (*R. solani* reduced by 99-100% and *Pythium* spp. reduced by 87-96%). Similarly, in the commercial trials conducted in 2009-10 and 2010-11 (see Section 4.3), MI:Pic reduced DNA concentrations of *Pythium* spp. (clade F) and *R. solani* AG 2.1 to equivalent levels as MB:Pic (e.g. Table 4.3).

Spatial analysis of DNA concentrations of *Pythium* spp. before and after fumigation in the commercial trial in 2010-11 (see Section 4.3) showed that MI:Pic reduced levels by an average of 95%, evenly across the treated block (Figure 4.7).

Table 4.2. Average levels of naturally occurring soil-borne pathogens (pg of DNA / g soil) in commercial strawberry runner blocks, pre- and post- fumigation treatment at Toolangi Victoria in 2008-09. There was no significant ($p \leq 0.05$) difference in DNA levels in soil between fumigant treatments.

Pathogen	**Untreated**	**MI:Pic (30:70) 500 kg / ha**			**MB:Pic (50:50) 500 kg / ha**			**MB:Pic (50:50) 375 kg / ha**		
		10 cm	30 cm	80 cm	10 cm	30 cm	80 cm	10 cm	30 cm	80 cm
Rhizoctonia solani (AG 3)	60.8*	0.7	0.0	0.0	0.2	0.7	0.2	0.5	16.5	0.0
Pythium spp. (cladeF)	634.1	95.5	4.8	1.3	44.0	6.3	6.0	81.0	50.0	12.5

*units = pg of DNA / g of soil

Table 4.3. Average levels of naturally occurring soil-borne pathogens (pg of DNA / g soil) in a commercial trial in the strawberry runner industry at Toolangi, Victoria in 2009-10. Values at 10 cm depth followed by different letters in each column are significantly different, where $p \leq 0.05$.

Treatment	***Rhizoctonia solani* AG 2.1**			***Pythium* spp. clade F**		
	10 cm	30 cm	80 cm	10 cm	30 cm	80 cm
MB:Pic (50:50) 500 kg /ha	1 b	1	0	7 b	2	0
MB:Pic (50:50) 250 kg / ha	0 b	0	0	7 b	4	6
MI:Pic (50:50) 500 kg / ha	1 b	0	0	8 b	3	0
MI:Pic (50:50) 350 kg/ha	0 b	0	0	5 b	0	0
Untreated	64 a	1	2	196 a	16	51

4.3.4 Weed emergence

Commercial trials consistently demonstrated the ability of MI:Pic to reduce weed emergence to the same level as MB:Pic. For example, in the commercial trials conducted in 2008-09 (see Section 4.3) the number of emerging weeds within ten × 1 m² quadrats per plot was recorded at 1 month after fumigation. Results showed that weed emergence was equivalent in the standard rate MB:Pic and MI:Pic treatments, and significantly less than in the lower rate MB:Pic treatment (Figure 4.8). The major

weeds that were not completely controlled by MI:Pic and the MB:Pic treatment included the hard-seeded legumes (*Trifolium* spp., *Melilotus* spp., *Medicago* spp., *Vicia* spp.). However the population density of these weeds was very low.

4.3.5 Runner certification

The Manager of the Victorian Strawberry Industry Certification Authority inspected strawberry plants growing in MI:Pic and MB:Pic-treated soils in all commercial trials. Plants in all blocks were deemed to have met the certification standards prescribed in the Victorian Runner Certification Scheme, and the Manager reported no differences in plant health standards or rates of 'rogueing'. No plants were suspected of soil-borne diseases and, consequently, no plants were sent away for pathogen testing. These results demonstrate the potential for MI:Pic to achieve the same certification standards for runners as MB:Pic under commercial settings.

4.3.6 Runner quality and fruit yields

Commercial trials conducted in the strawberry fruit industry showed that runners produced in soils treated with MI:Pic yielded equally to those produced in MB:Pic-treated soils.

In 2008, samples of strawberry runners (var. Albion) grown in soils at Toolangi, Victoria treated with MI:Pic (30:70) at 500 kg/ha and MB:Pic (50:50) at 500 kg/ha were harvested. These runners were planted in three commercial trials (Millgrove, Wandin North and Seville, Victoria) in the strawberry fruit industry in soils treated with Pic at 400 kg/ha. Trials were conducted as randomised complete block designs with three replicates. There was a consistent trend towards higher commercial fruit yields from runners produced in MI:Pic soils, compared with those from MB:Pic soils, but this was not statistically significant (Fig 4.9). These trials provide evidence that MI:Pic does not adversely affect runner quality compared with MB:Pic.

4.4 Discussion

Results from commercial trials conducted over four years in this project support previous small-scale trials (Mann et al., 2005; 2008; Mattner et al., 2005; 2008) showing that the efficacy of MI:Pic mixtures for soil disinfestation in the runner industry is equivalent to MB:Pic. Data consistently showed that MI:Pic controlled pathogens and weeds, and increased runner yields to the same level as MB:Pic. Unlike other alternative fumigants trialled in the runner industry (e.g. 1,3-D:Pic, metham sodium), there was no evidence of phytotoxicity occurring in runner crops in MI:Pic-treated soils. Furthermore, certification standards and runner quality in the fruit industry were maintained under MI:Pic regimes compared with MB:Pic.

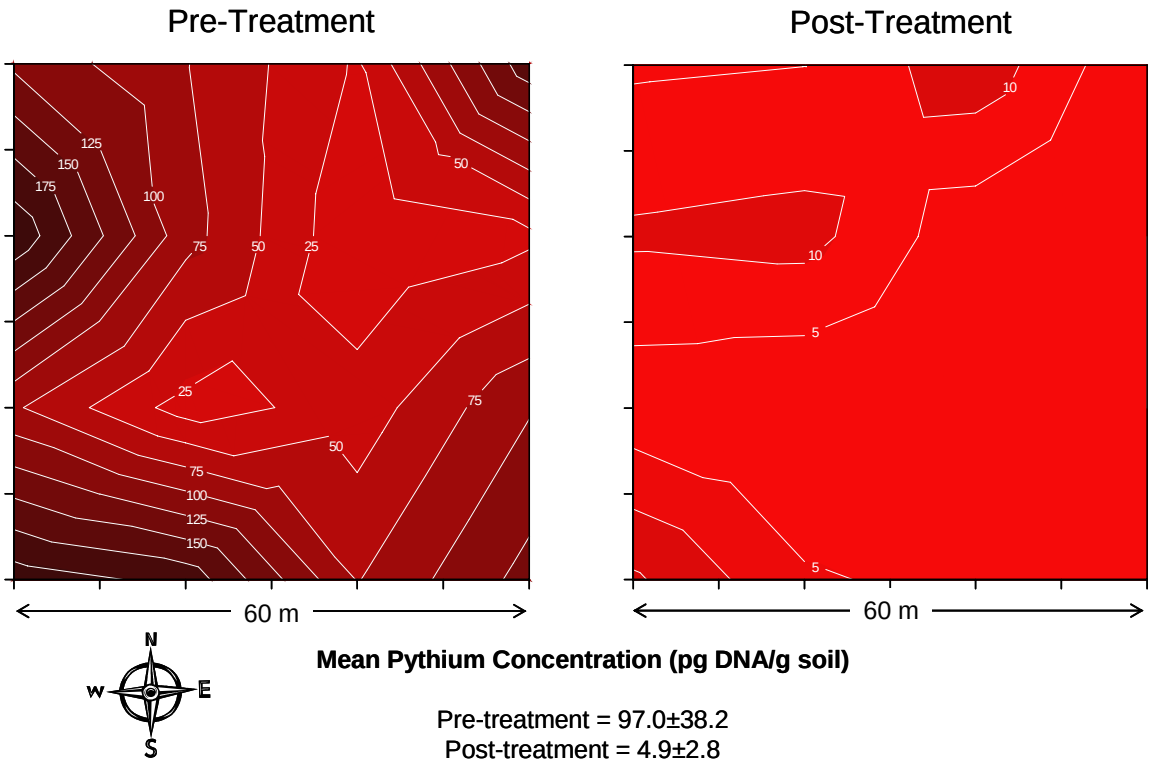


Figure 4.7. Spatial variation of *Pythium* clade F concentrations in soil, pre- and post-treatment (7 days), in a block fumigated with MI:Pic (50:50), 500 kg/ha at Toolangi , Victoria in 2010-11.

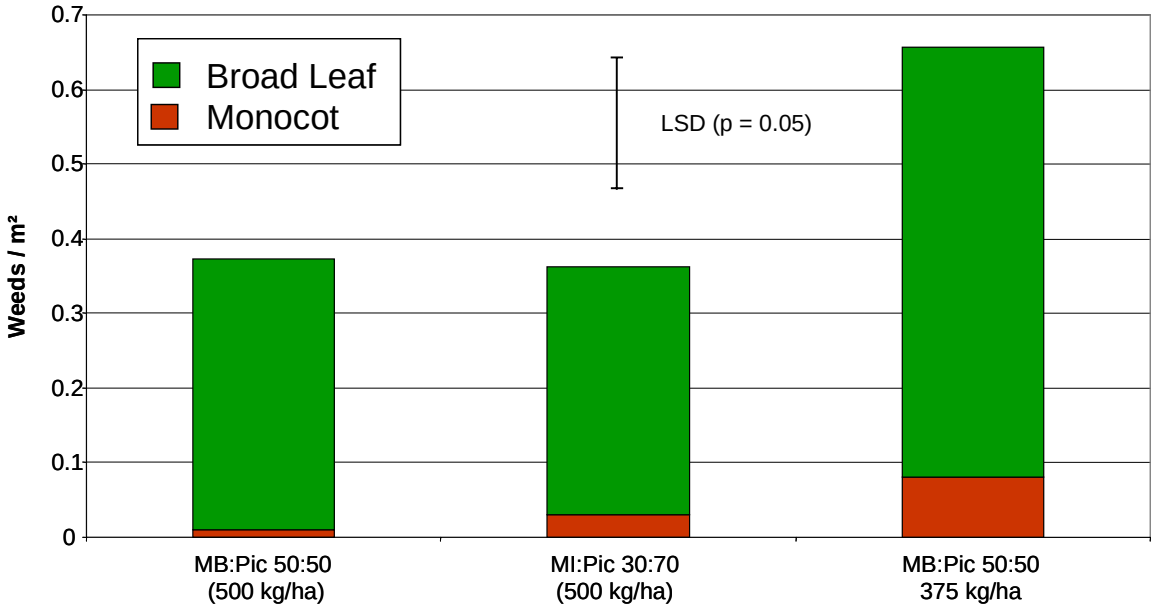


Figure 4.8. Weed emergence in soils treated with various fumigants in commercial trials at Toolangi, Victoria in 2008-09. The bars is the least significant difference where p = 0.05.

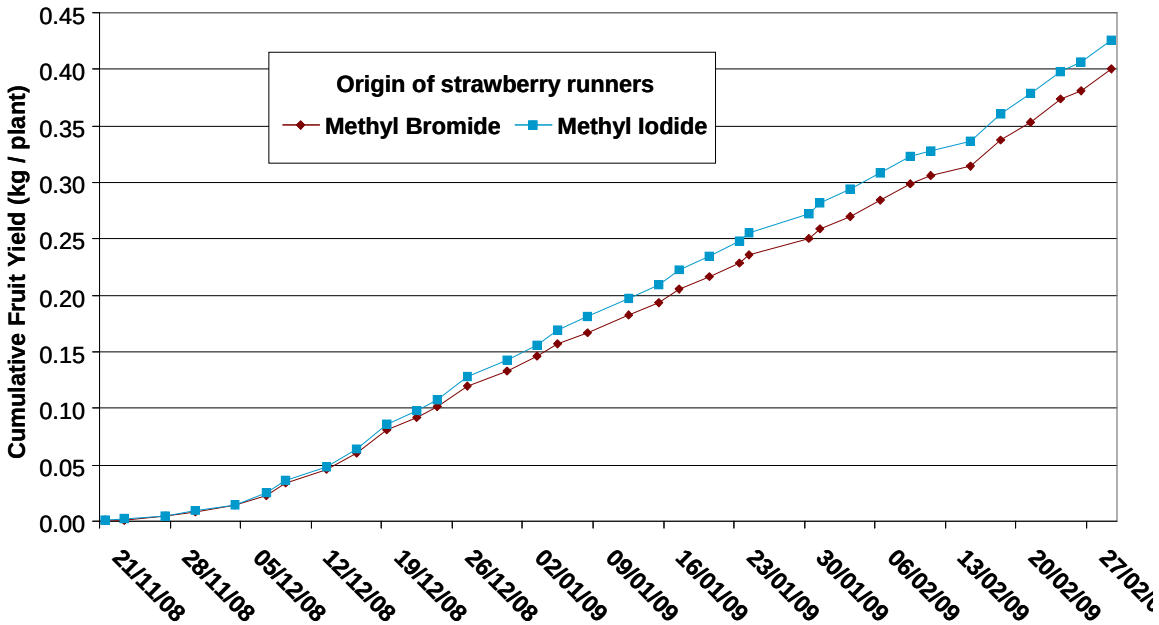


Figure 4.9. Cumulative fruit yields of runners produced in soils treated with MI:Pic and MB:Pic in the nursery in a field trial at Millgrove, Victoria. There was no significant difference between treatments.

Overall, these results suggest that the risk of biosecurity standards failing in the strawberry industry following a transition from MB:Pic to MI:Pic for soil disinfestation in the runner industry is negligible. The implication is that the risk of soil-borne pathogens and weeds being transported to strawberry fruit growers in strawberry runners (or in the rhizosphere surrounding runners) grown in MI:Pic-treated soils is equivalent to MB:Pic. Technical data from this and previous projects has been delivered to: (1) the Office of the Chief Plant Protection Officer to support decision-making on the interstate market access of runners grown in MI:Pic soils, (2) strawberry runner authorities to allow decisions on the approval and inclusion of MI:Pic in Australian certification schemes, and (3) the APVMA to support the possible registration of MI:Pic in the runner industry. Results showed that MI:Pic is currently the best placed alternative fumigant to replace MB, however its recent withdrawal means it will not be registered in the short term to assist the strawberry runner industry to phase-out MB. The future of methyl iodide is uncertain.

5. Disease and Pathogen Thresholds (Activity 2)

5.1 Introduction

The biosecurity and market access of the Australian strawberry industry is under threat from current (e.g. methyl bromide, MB) and possible future (e.g. chloropicrin, Pic, is listed for review) withdrawals of soil fumigants. In Australia and worldwide, most strawberry nurseries produce transplants under phytosanitary controls or 'certification', where regulations mandate the use of soil fumigants (Porter et al., 2006b). In Australia, strawberry transplants go through four generations of multiplication before delivery to fruit growers, with these generations requiring soil fumigation and disease inspection to meet certification. The Victorian strawberry nursery industries provide up to 85% of runner transplants to Australian fruit growers. All the runner transplants are grown under a certification scheme in order to provide disease freedom for certain pathogens. Many fungal pathogens are not part of certification as they are too widespread and difficult to eliminate from soils therefore pathogen tolerances would be useful for industry. Strawberry runner transplants go through four generations of multiplication before delivery and to meet certification regular disease inspections and monitoring for soil and plant borne diseases. For some states, runner transplants are sold as bare roots with either leaves on or off. Most of leaf pruning is done during harvesting for the leaf off runner transplants.

Worldwide, strawberry certification schemes have reduced the incidence of soil-borne pathogens and viruses in transplants, and contributed to a 300% increase in fruit yields since the 1940s (Porter et al., 2006a). Presently, the soil fumigant MB/Pic is approved for use as the major biosecurity tool for minimising the risk of transporting soil-borne pathogens (viz. *Phytophthora cactorum*, *Verticillium dahliae*, *Rhizoctonia* spp., *Pythium* spp., *Fusarium* spp.) in strawberry transplants sent around Australia. Regulatory authorities in some Australian states only grant certification or accept entry of strawberry transplants if they are produced in soils treated with MB/Pic. For example, the conditions of the Victorian Strawberry Runner Certification Scheme state: '3.1.6: The site must be fumigated with a registered chemical approved by the Victorian Strawberry Industry Certification Authority (VSICA). Currently, VSICA approves soil fumigation with a 50:50 mixture of methyl bromide and chloropicrin applied at a rate of 500 kg/ha under plastic film'. An Act of State Parliament underpins the conditions of the certification scheme (Plant Health and Plant Products Act, 1995).

Due to these regulatory challenges and difficulties in adopting registered alternative fumigants (see below), the southern Australian strawberry nursery industry have applied annually, since 2005, for critical-use nominations to retain 29.79 tonnes of MB for soil disinfestation purposes. In this way, the phase-out of MB is not only challenging the industry to find alternatives, but also to prove that alternatives meet certification and biosecurity standards. Research conducted in Australia over the last 10 years has identified fumigant and non-fumigant alternatives to MB for strawberry transplant production, and has recently commenced determining pathogen threshold levels that meet certification standards for transplants. This chapter reviews some preliminary studies to assist with determination of threshold tolerances for three key pathogens, *Gnomoniopsis fructicola, Pythium* spp., *Rhizoctonia solani AG* 2.1. Issues surrounding *Phytophthora cactorum* are also discussed.

5.2 Materials and methods

5.2.1 *Gnomoniopsis* surveys

Incidence survey procedures described by de Boer *et al*. (1996) were used to study *Gnomoniopsis fructicola*. 100 runners each from generations 2 and 3 (see Section 3.3) were sampled from cold storage 12-16 weeks after harvest. 100 runners from generation 4 (see Section 3.3) were sampled immediately following harvest. Varieties sampled included Festival, Albion and Camarosa. Surface sterilised petiole segments were plated onto potato dextrose agar amended with achromycin (PDA+A) and incubated at 23^{O}C. Morphological characteristics were used to identify *G. fructicola* (e.g. production of pycnidia).

5.2.2 Effectiveness of MB:Pic against *Gnomoniopsis*

Current preventative measures for *Gnomoniopsis* in the runner industry include long rotations and fumigation with MB:Pic to kill inoculum surviving in crop debris, and fortnightly fungicidal sprays in the growing crop with prochloraz. However, the incidence of *Gnomoniopsis* in certified runners grown under MB:Pic fumigation and prochloraz application has not been adequately benchmarked.

Infected strawberry plant material was collected from crop debris in growers' paddocks and buried in trial plots prior to fumigation with MB:Pic (50:50) at 500 kg/ha. Plant material was also buried in untreated plots as a control treatment. Five days after treatment plant material was recovered, surface sterilised and plated onto ¼ strength PDA + A media. *G. fructicola* was identified using microscopy methods.

5.2.3 Pathogen thresholds for *Pythium* spp. in soil

Concentrations of *Pythium* clade f (using SARDI's test) were studied throughout the project in soils treated with MB:Pic 50:50 (500 kg/ha) and untreated (see chapter 4 for method). *Pythium* clade f contains many of the *Pythium* species pathogenic to strawberries (Levesque and de Cock 2004). Concentrations in soil across 4 seasons were averaged to establish the threshold level for this pathogen is soil using the MB:Pic treatment as the benchmark.

5.2.4 Pathogen thresholds for *Phytophthora* spp. in plant

Following harvest, the crowns of runners sampled from the commercial trials described in Chapter 4 were cut open and inspected for symptoms of crown rot (caused by *Phytophthora cactorum*). Isolations onto Phytophthora selective media (PARP) were made from a sample of crowns from each trial. In the largest trial, a total of 1400 runners were sampled from commercial blocks treated with MI:Pic 30:70 (500 kg/ha) or MB:Pic 50:50 (500 kg/ha).

5.3 Results

5.3.1 *Gnomoniopsis* surveys

Surveys conducted in 1990 detected intermediate levels (averaging 50%) of *G. fructicola* in runners from all stages of the multiplication scheme. The incidence of *G. fructicola* in runners from the multiplication scheme was lower in surveys conducted during 2009-2011 compared to 1990 (Table 5.1). Adoption of hygiene and fungicide regimes (prochloraz) during the 1990s was largely responsible for this reduction.

Table 5.1 Incidence of Gnomoniopsis (%) found in runners sampled from cold storage and grower sheds.

Year	Nucleus (Generation 1)	Foundation (Generation 2)	Mother (Generation 3)	Commercial (Generation 4)
1990	-	45	66	40
2009-2010	-	0	33	12
2010-2011	0	8	12	22

5.3.2 Effectiveness of MB:Pic against *Gnomoniopsis*

No *G. fructicola* was recovered from material buried in the MB:Pic-treated plots but 100% recovery was made from untreated control plots (Table 5.2). This indicates MB:Pic is an effective pesticide for *Gnomoniopsis* and can eradicate the organism from infected crop residues in soil when used as a preplant fumigant.

Table 5.2 Recovery of *Gnomoniopsis* from strawberry plant residues buried in soil and treated with MB:Pic.

Treatment	(%) recovery of *Gnomoniopsis*
MB:Pic (50:50) 500 kg/ha	0
Untreated	100

5.3.3 Pathogen thresholds for *Pythium* spp. in soil

Average concentrations of *Pythium* clade f in soils treated with MB:Pic over a four year period were 3.9 pg/DNA g soil (Fig 5.1). This concentration may provide a threshold level for this pathogen in soil at planting that is acceptable for growth and certification of strawberry runners.

5.3.4 Pathogen thresholds for *Phytophthora* spp. in plant

The incidence of crown rot associated with the isolation of *P. cactorum* was generally less than 1%, so significant differences between treatments could not be detected. Therefore, before pathogen thresholds can be determined for *P. cactorum*, high throughput quantitative molecular techniques will need to be developed in order to process much larger sample numbers of runners.

5.4 Discussion

In the short-term, it was hoped that the Australian strawberry nursery industry could use effective alternative fumigants such as MI/Pic provided certification authorities accepted their use. However, now that the registrants have decided not to register methyl iodide, longer-term solutions must be considered. Industry may need to consider a future without soil fumigants, which may include soil-less production systems and non-pesticide methods of soil disinfestation. Threshold levels for soil-borne pathogens should be determined for certification standards, but our results have demonstrated the need for more sensitive high-throughput diagnostic tools that can assist with this. This will allow industry to make informed decisions on future production systems, and enable regulators to assess the biosecurity and market access risks in support of these changes.

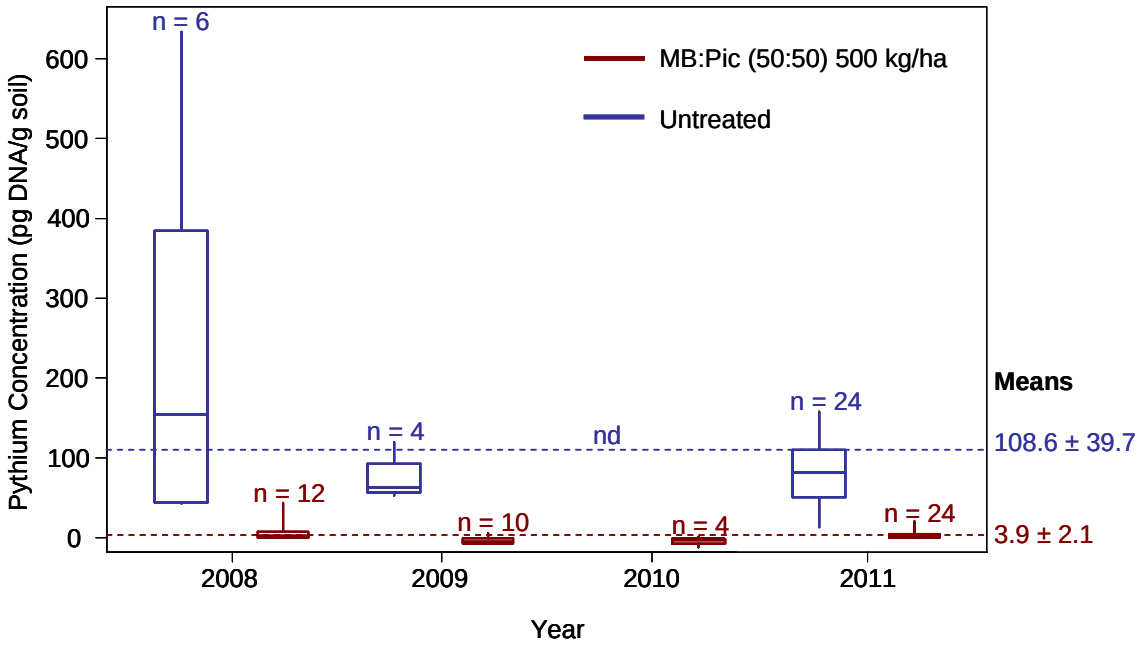


Figure 5.2. Boxplot of *Pythium* clade f concentrations at a depth of 10 cm in MB:Pic and untreated soils taken 4 weeks after treatment at various times throughout the project from locations within the Toolangi Plant Protection District. Clade f contains most of the *Pythium* spp. that are pathogenic to strawberry.

6. Barrier Films and Drip Fumigation (Activity 3)

6.1 Introduction

This chapter reports on trials conducted from 2007 to 2010 in New Zealand and Australia to determine if drip application (through trickle irrigation lines) and barrier films (plastic tarpaulins that are more impermeable to fumigants than standard plastic films) could improve the efficacy of fumigant alternatives to methyl bromide / chloropicrin (MB:Pic) formulations. Trials were conducted in the strawberry fruit industry with drip fumigants, and in the strawberry runner industry with impermeable films.

The research in NZ and Australia focused on four main areas: comparing alternative fumigants for strawberry fruiting beds; comparing drip application of fumigants through the trickle irrigation system with application by standard shank injection; alternative fumigants for the strawberry runner industry; and the use of barrier films to allow reduced fumigant application rates in the runner industry.

6.2 Materials and Methods

6.2.1 NZ Strawberry fruit trials

Roselea trial 2007/08

A pilot drip fumigation trial was established at Roselea in late April/early May 2007, with applications of the following treatments in 20-m-long beds:

1. Untreated control
2. Chloropicrin – drip application
3. Chloropicrin – shank injection
4. Iodomethane (methyl iodide:chloropcrin 50:50) – drip application
5. Iodomethane (methyl iodide:chloropcrin 50:50) – shank injection
6. Telone C35 (1,3-dichloropropene:chloropicrin 65:35) – drip application
7. Telone C35 (1,3-dichloropropene:chloropicrin 65:35) – shank injection.

Shank injection treatments were applied using standard practices, i.e. injected into the soil via shanks as the beds were formed and plastic was laid. There was a single shank, set at a depth of 25-30 cm, at the centre of the bed. Beds were rounded, with a base width of approximately 60 cm.

For drip application, beds were formed and plastic was laid without gas injection. One or two days later, fumigants were applied through the irrigation tape. This was done using the experimental injection system illustrated in Figure 6.1. The respective fumigants were mixed with an emulsifier (Nutrapic®) so that they would dissolve in water, and then gradually injected into the irrigation system as the trial rows were irrigated over a two-hour period. The total amount of fumigant applied per unit area was the same as for shank-injected treatments (60 g/m^2).

Five strawberry varieties (‘Camarosa’, ‘Camino Real’, ‘Gaviota’, ‘Pajaro’ and ‘Ventana’) were planted four weeks after fumigation. Each trial plot was 1.4 m long,

Figure 6.1. Drip fumigant application apparatus used in experimental strawberry plots.

and consisted of seven, nine or 11 plants, depending on the variety. There were from three to six replicates of each treatment for each variety.

Wetting pattern
Before fumigants were applied via drip, the wetting pattern of the beds was investigated by running irrigation through spare beds and excavating sections at 30-min time intervals. Beds were photographed at each of these times, and percentage water content at various points in the bed was measured using a Hydrosense™ soil moisture monitor.

Gas distribution
To determine fumigant movement within beds, Gastec® detector tubes were used to measure fumigant gases in the soil. Readings were taken at various points 4, 24 and 48 h after fumigant application. Readings were also taken at weekly intervals to determine the longevity of the respective fumigants in the soil.

Pathogen and weed kill
Small mesh bags containing soil naturally infested with *Phytophthora cactorum* and weed seeds (clover and ryegrass) were buried in beds either before (drip application) or immediately after (shank injection) fumigant application. These bags were recovered three weeks after fumigation, and survival of *Phytophthora* and weed seeds was determined in laboratory assays.

Plant growth parameters
In spring (October 2007), plant growth was assessed by measuring the average canopy height of each plant. After the end of the fruiting season (January/February 2008), final assessments of canopy growth, plant health and feeder root health were made. Plant canopy health was scored on a 0 to 4 scale, where 0 was healthy and 4 was dead. Root health was scored on a scale from 0 to 4 where 0 = all feeder roots healthy, 1 = 25% diseased, 2 = 50% diseased, 3 = 75% diseased, 4 = 100% diseased.

Fruit were harvested weekly throughout the fruiting season from mid October 2007 until early January 2008, and total fruit weight from each plot was assessed. Plots were covered with bird netting throughout the harvest season to minimise fruit losses.

Roselea trial 2008/09

A follow-up drip fumigation trial was established at Roselea in April 2008. Chloropicrin, TeloneC35 (1,3 dichloropropene:chloropicrin 65:35), TeloneC60 (1,3 dichloropropene:chloropicrin 40:60), Iodomethane (methyl iodide:chloropicrin 50:50) and Fumasol (metam sodium) were applied to 20-m beds via either shank injection or drip application through the irrigation tape, as described for the 2007/08 trial above. Pre-wetting of beds and differing volumes of water during drip application were also tested, to determine whether these factors affect fumigation effectiveness. Treatments applied are shown in Table 6.1.

Pre-wetting of beds, where appropriate, was done by irrigating through the T-tape for 1 h, applying a total of 5 L/m bed length, 4 days before fumigant application. For most drip treatments, the fumigants were applied over a 1.5 h period, in a water volume of 7.5 L/m bed length. The same amount of fumigant was applied in a water volume of 12.5 and 5 L/m for 'High' and 'Low' volume treatments, respectively. This was done by varying the injection rate of the fumigant into the water flow, and changing the time of irrigation accordingly.

Five strawberry varieties ('Camarosa', 'Camino Real', 'Gaviota', 'Pajaro' and 'Ventana') were planted, five weeks after fumigation. Each 'plot' was 1.0 m long, and consisted of five, seven or nine plants, depending on the variety. There were five replicates of each treatment for each variety.

Pathogen and weed kill

To test fumigant efficacy, small nylon mesh bags containing soil naturally infested with *Phytophthora cactorum* plus ryegrass seeds were buried in various parts of beds either before (drip application) or immediately after (shank injection) fumigant application. In four replicate beds of every treatment, bags were placed in each of the following three positions:

- Top: 5 cm deep, mid way between the centre of the bed and the shoulder
- Shoulder: 5 cm deep directly in from the shoulder of the bed
- Bottom corner: 5 cm in from the bottom outside corner of the bed, slightly above the final soil level outside the bed.

All bags were recovered three weeks after fumigation, and the survival of *Phytophthora* and ryegrass seeds was determined in laboratory assays.

Plant growth parameters

In spring (October 2008), plant growth was assessed by measuring the average canopy height of each plant. After the end of the fruiting season (January/February 2009), final assessments of canopy height and plant health were made. Plant canopy health was scored on a 0 to 4 scale, where 0 was healthy and 4 was dead.

Table 6.1. Treatments applied in the fumigation trial at Roselea in the 2008/09 season. Fumigants were applied via either shank injection or through the drip irrigation tape. Differing volumes of water were used in the drip application of some products, and some plots were pre-wetted.

Treatment abbreviation	Fumigant	Rate (g/m^2)	Application technique	Water volume[a]	Pre-wetting of bed
Chl -Drip	Chloropicrin	65 g/m^2	Drip	7.5	No
Chl -Drip-HIGH vol	Chloropicrin	65 g/m^2	Drip	12.5	No
Chl -Drip-LOW vol	Chloropicrin	65 g/m^2	Drip	5	No
Chl –Drip pre-wet	Chloropicrin	65 g/m^2	Drip	7.5	Yes
Chl -Shank	Chloropicrin	65 g/m^2	Shank	-	No
Fum-Drip	Fumasol	80 ml/m^2	Drip	7.5	No
Iodo-Drip	Iodomethane	50 g/m^2	Drip	7.5	No
Iodo-Shank	Iodomethane	50 g/m^2	Shank	-	No
TC35-Drip	TeloneC35	65 g/m^2	Drip	7.5	No
TC35-Shank	TeloneC35	65 g/m^2	Shank	-	No
TC60-Drip	TeloneC60	65 g/m^2	Drip	7.5	No
TC60-Drip pre-wet	TeloneC60	65 g/m^2	Drip	7.5	Yes
TC60-Shank	TeloneC60	65 g/m^2	Shank	-	No
Untreated	Nil	-	-	-	No

[a] water volume (L/m bed length) used during application of fumigant via drip irrigation tape

Fruit were harvested weekly throughout the fruiting season from mid October until late December 2008, and total fruit weight from each plot was assessed. Plots were covered with bird netting throughout the harvest season to minimise fruit losses.

Roselea trial 2009/10

In an attempt to resolve differences between results from 2007 and 2008 trials at Roselea, a further drip versus shank fumigant application trial was established in April 2009. It compared drip or shank-applied chloropicrin, TeloneC60 (1,3 dichloropropene:chloropicrin 40:60) or iodomethane (methyl iodide:chloropicrin 50:50), drip- applied Fumasol (metam sodium), and untreated controls, with five replicate 20-m beds of each treatment. Application techniques were the same as those outlined for the 2007 and 2008 trials above, with fumigant rates the same as in the 2008 trial. All drip-applied fumigants were applied over a 1.5-h period in 7.5 L of water/m of bed.

In some other countries (e.g. Spain and Italy), drip application is used to re-fumigate existing strawberry beds for use a second year, rather than cultivating and reforming a new bed. In an effort to test the potential for this treatment in New Zealand, some beds were retained from the previous season's trial. Strawberry plants were killed with herbicide and removed before re-fumigation with either chloropicrin, iodomethane or TeloneC60 injected through the drip irrigation system as described above. There were four replicate beds of each fumigant, with comparable beds left as untreated controls.

Five strawberry varieties ('Camarosa', 'Camino Real', 'Gaviota', 'Pajaro' and 'Ventana') were planted 5 weeks after fumigation.

Plant back
To test plant-back time following fumigation, 'Pajaro' plants were planted at weekly intervals, starting one week after fumigation. There were four replicate plots of six plants in each fumigation treatment at each plating time. Plant mortality and health was assessed in September, 4-5 months after planting, and fruit from each plot was weighed throughout the harvest season.

Pathogen and weed kill
In four replicate beds of every treatment, small nylon mesh bags containing soil naturally infested with *Phytophthora cactorum*, plus ryegrass and clover seeds, were buried in either the shoulder or bottom corner of beds either before (drip application) or immediately after (shank injection) fumigant application. In both positions, bags were approximately 5 cm beneath the plastic.

All bags were recovered two weeks after fumigation, and survival of *Phytophthora* and ryegrass plus clover seeds was determined in laboratory assays.

Plant growth parameters
Fruit were harvested weekly from mid October until late December 2009, and total fruit weight from each plot was assessed. At the end of the fruiting season (February 2010), plant canopy health and height were assessed. Plant canopy health was scored on a 0 to 4 scale, where 0 was healthy and 4 was dead.

6.2.2 Australian strawberry fruit trial

Millgrove trial 2008/09

A field trial was established on a commercial strawberry fruit farm in Millgrove, Vic comparing shank and drip applied fumigants for soil disinfestation. Fumigant treatments are listed in Table 6.2. Fumigation occurred in May 2008 and planting (var. Albion) in June 2008. The trial was designed as a randomised complete block design with three blocks.

Fumigant residues
Soil probes were constructed from a 1.6 mm internal diameter (i.d.) hollow copper tube protected by a 6.44 mm i.d. brass tube attached to a 50 cc syringe with Teflon tubing and a latex hose. Probes were inserted following fumigation with methyl iodide:chloropicrin 50:50 (IM) through the low-density polyethylene barrier film into the middle of the bed at a depth of 20 cm. Gaseous fumigant residues of IM were collected on Gastec detector tubes (121L Benzene) by drawing up to 500 mL of soil air. Once a colour change was noted on the detector tube air sampling was terminated. The

Table 6.2. Treatments applied in the fumigation trial at Millgrove in the 2008/09 season. Fumigants were applied via either shank injection or through the drip irrigation tape.

Treatment	Code	Rate	Method of Application
Methyl Iodide : Chloropicrin (50:50)	IM:Pic	350 kg / ha	Shank Injection
Chloropicrin	Pic	400 kg / ha	Shank Injection
Chloropicrin & Metham Sodium*	Pic / MS	400 kg / ha & 300 L/ha	Shank Injection / Drip
Methyl Bromide Chloropicrin (50:50)	MB:Pic	500 kg / ha	Shank Injection
Methyl Iodide : Chloropicrin (50:50)	IM:Pic EC	350 kg / ha	Drip Irrigation System
Chloropicrin	Pic EC	400 kg / ha	Drip Irrigation System
Chloropicrin & Metham Sodium*	Pic EC / MS	400 kg / ha & 300 L/ha	Drip Irrigation System
Metam Sodium	MS	800 L / ha	Drip Irrigation System
Untreated			

* Applied via the drip irrigation system 1 week after initial chloropicrin application

volume of air drawn was recorded, along with the concentration (ppm) of IM indicated by the detector tube. Assessments were conducted 1, 7, 14 and 21 days after fumigation.

Vegetative growth (in season)
The leaf number and length area were measured on 10 randomly selected plants per replicate within each treatment at 48 days after planting.

Efficacy (weeds)
Weed emergence was determined 48 days after planting by assessing four planting holes in the middle of each plot. All weeds within the holes were identified and counted. All data were expressed on a weeds / planting hole basis.

Yields
Yields were taken throughout the early fruiting period (ie 1 – 3 times per week, November 08 – December 08). Commercial pickers were used to harvest marketable fruit. Yields were expressed as the fresh weight of marketable fruit per plant.

6.2.3 NZ Strawberry runner trial

Katikati trial, 2008

The Katikati trial was established in late August 2008, when soil temperatures were between 12 and 18ºC in the top 20 cm. Fumigants used were iodomethane (methyl iodide:chloropicrin 50:50), applied at rates of 250 (low) or 450 (high) kg/ha, or TeloneC60 (1,3 dichloropropene:chloropicrin 40:60), applied at rates of 300 (low) or 500 (high) kg/ha.

Beds (25 m long) were fumigated with either TeloneC60, Iodomethane, or left untreated. The first 13 m was treated with the high fumigant rate, the remaining 12 m at low rate (or vice-versa, randomly). Standard low-density polyethylene was applied to

the whole bed during fumigation; within 30 minutes, VIF film was manually inserted in two 5-m strips within the bed (one at the 'low rate' end, one at the 'high rate' end), and the polyethylene then removed from these plots. Thus, each bed was split into four subplots. Treatments were:

MI P H - Methyl iodide/chloropicrin50:50, high rate, Polyethylene
MI P L - Methyl iodide/chloropicrin50:50, low rate, Polyethylene
MI V H - Methyl iodide/chloropicrin50:50, high rate, VIF
MI V L - Methyl iodide/chloropicrin50:50, low rate, VIF
T P H - Telone C60, high rate, Polyethylene
T P L - Telone C60, low rate, Polyethylene
T V H - Telone C60, high rate, VIF
T V L - Telone C60, low rate, VIF
Un - Unfumigated control.

There were six replicate subplots of each treatment combination, laid out in a randomised block design across the site. The plastic was removed after 7 days. Untreated areas between plots were covered with plastic to minimise plot contamination from untreated soil. This plastic maintained its integrity for about 4 months after fumigation. Following fumigation, all soil work in the block was done by hand or with a small rotary hoe, with care taken to minimise cross contamination of soil between plots.

'Camarosa' strawberry plants from the Elite bed were planted approximately 7 weeks after fumigation. Double planting was done (0.9 m centres), with the aim of removing every second plant for productivity assessment in January/February.

Buried pathogen and weed kill
Immediately after fumigation, mesh bags containing approximately 20 g of *Phytophthora cactorum*-infested field soil plus ryegrass and clover seeds were buried at 5 cm and 20 cm depths, at one point within each plot.

Bags were dug up after 7 days (just before lifting the plastic), assayed for weed and *Phytophthora* survival, and compared with similar bags in untreated controls.

Weed emergence
Weed counts were made in all plots after 7, 12 and 16 weeks. After each assessment, weeds were removed by a combination of cultivation, manual weeding and herbicides.

Runner growth and yields
Plant growth was first assessed in early February 2009, with the excavation of every second mother plant and attached daughters. Rooted runners were counted and all plants were weighed. At the final harvest in May 2009, all plants were lifted. Total strawberry biomass was measured, and marketable plants were counted and weighed. Notes were also made on root health.

Fruit yield of runners
Representative plants of similar size (10-12 mm crown diameter), from all runner bed plots, were planted into Telone C60-fumigated beds in the experimental fruit garden at Roselea. Plant health and fruit yield was monitored throughout the 2009/10 season, with the final assessments of plant health made in February 2010.

6.2.4 Australian strawberry runner trial

Toolangi trial, 2008/09

A field trial was conducted in 2008-09 at Toolangi. The trial was conducted as a randomised split-plot design with 4 blocks. Fumigant treatments, all applied by shank injection, were the main plots and film treatments the split plots (Table 6.3). The film treatments were standard low density polyethylene (35 μm) and Bromostop® impermeable film, which is a polyamide / polyethylene laminate. The plots were fumigated in October 2008. The film remained in place for 7 days (slightly longer than commercial standards). Plots were planted 4 weeks after fumigation with mother plants spaced 50 cm apart (var. Gaviota). Yield assessments were taken in June 2009 as described previously.

Table 6.3. Treatments applied in the fumigation trial at Toolangi in the 2008/09 season. Fumigants were applied via either shank injection.

Fumigant Treatment	Application Rate	Film Treatment
methyl bromide :chloropicrin (50:50)	50 g/m²	Low density polyethylene (LDPE)
methyl bromide :chloropicrin (50:50)	50 g/m²	Bromostop barrier film (VIF)
methyl bromide :chloropicrin (50:50)	25 g/m²	Low density polyethylene (LDPE)
methyl bromide :chloropicrin (50:50)	25 g/m²	Bromostop barrier film (VIF)
methyl iodide :chloropicrin (50:50)	50 g/m²	Low density polyethylene (LDPE)
methyl iodide :chloropicrin (50:50)	50 g/m²	Bromostop barrier film (VIF)
methyl iodide :chloropicrin (50:50)	35 g/m²	Low density polyethylene (LDPE)
methyl iodide :chloropicrin (50:50)	35 g/m²	Bromostop barrier film (VIF)
methyl iodide :chloropicrin (50:50)	17.5 g/m²	Low density polyethylene (LDPE)
methyl iodide :chloropicrin (50:50)	17.5 g/m²	Bromostop barrier film (VIF)
untreated	-	Low density polyethylene (LDPE)

6.3 Results

6.3.1 NZ Strawberry fruit trials

Roselea trial 2007/08

Wetting pattern

It was determined that for the Roselea soil, 1.5 – 2 h of irrigation at 8-10 psi was adequate to wet approximately two-thirds of the bed (Figures 6.2 and 6.3). This was the irrigation scheme used to apply drip fumigants

Figure 6.2. Wetting pattern in strawberry beds during drip irrigation at Roselea in 2007. Beds were cut and photographs taken at times of 30 min (top), 1 h (mid) and 2 h (bottom) after the commencement of irrigation.

Gas distribution

Fumigant gases moved throughout beds with both drip and shank application for all three fumigants tested, although the rate of distribution differed with the different gases (Figures 6.4 to 6.6). Four hours after application via drip, all three fumigants showed the highest gas concentration at the centre of the bed, between 20 and 30 cm depth, reflecting the depth at which irrigation water was at its highest concentration (Figure 6.3). For shank injection, after 4 h the highest fumigant concentration was at approximately 30 cm deep, reflecting the injection depth. Within 24 h, iodomethane had dispersed thoroughly and evenly throughout the bed, particularly following drip application. With chloropicrin and Telone, the gases were still pooled near the application point after 24 h, although some gas had dispersed throughout the bed. By 48 h, both Telone and chloropicrin had dispersed more evenly throughout the bed, although there was still a slight pooling near the application point with shank-applied chloropicrin and drip-applied Telone. For all three products, when the fumigant was drip-applied the gas moved beyond the wetted zone.

Iodomethane was effectively gone from the soil within two weeks, with negligible detectable levels at this time. Soil residence time for chloropicrin was between three and four weeks, and for Telone it was four to five weeks.

Pathogen and weed kill

Mortality of *Phytophthora cactorum* and weed seeds was close to 100% in all fumigated plots, regardless of the fumigant or the application method. This contrasted with close to 100% survival in unfumigated plots.

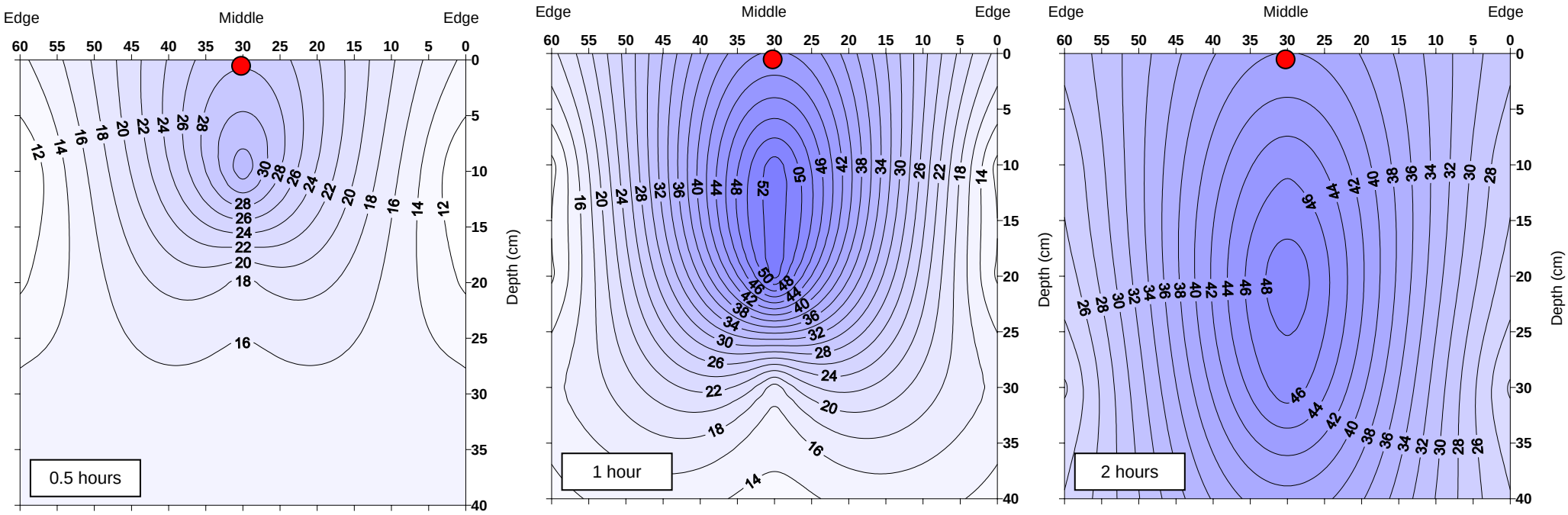


Figure 6.3. Contour plots of water concentration/distribution (%) in strawberry beds at Roselea, April 2007, from soil moisture probe readings taken at various intervals following the commencement of drip irrigation. Darker regions indicate higher water concentrations. The drip tape was on the surface at the centre of the bed (red circle).

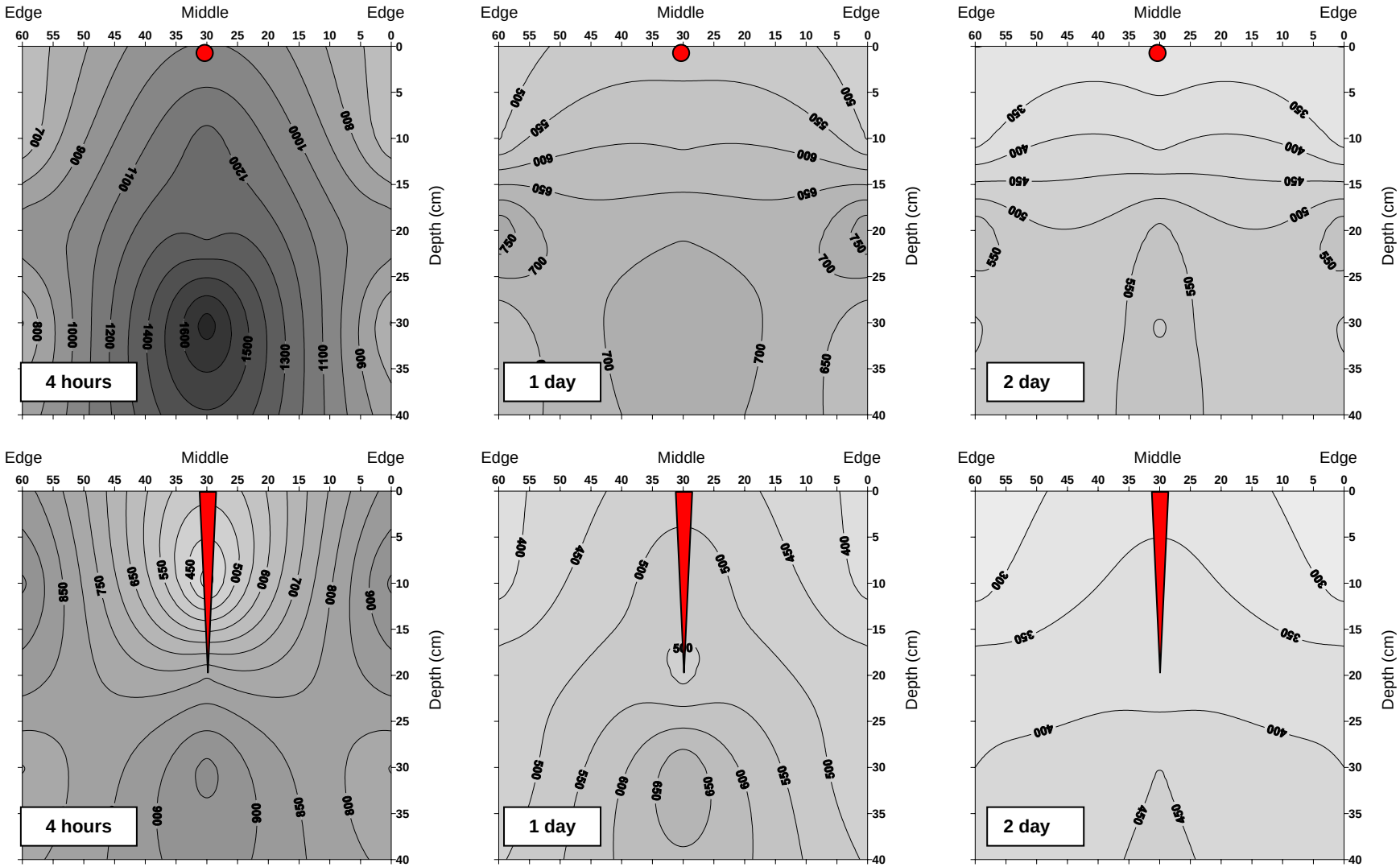


Figure 6.4. Contour plots, showing the lateral and vertical distribution of iodomethane through strawberry beds, following drip fumigation (top) and shank fumigation (bottom) - 4 h, 1 day and 2 days after application (red circles represent drippers at the soil surface, red triangles represent shank position). Darker regions indicate higher fumigant gas concentration in the soil.

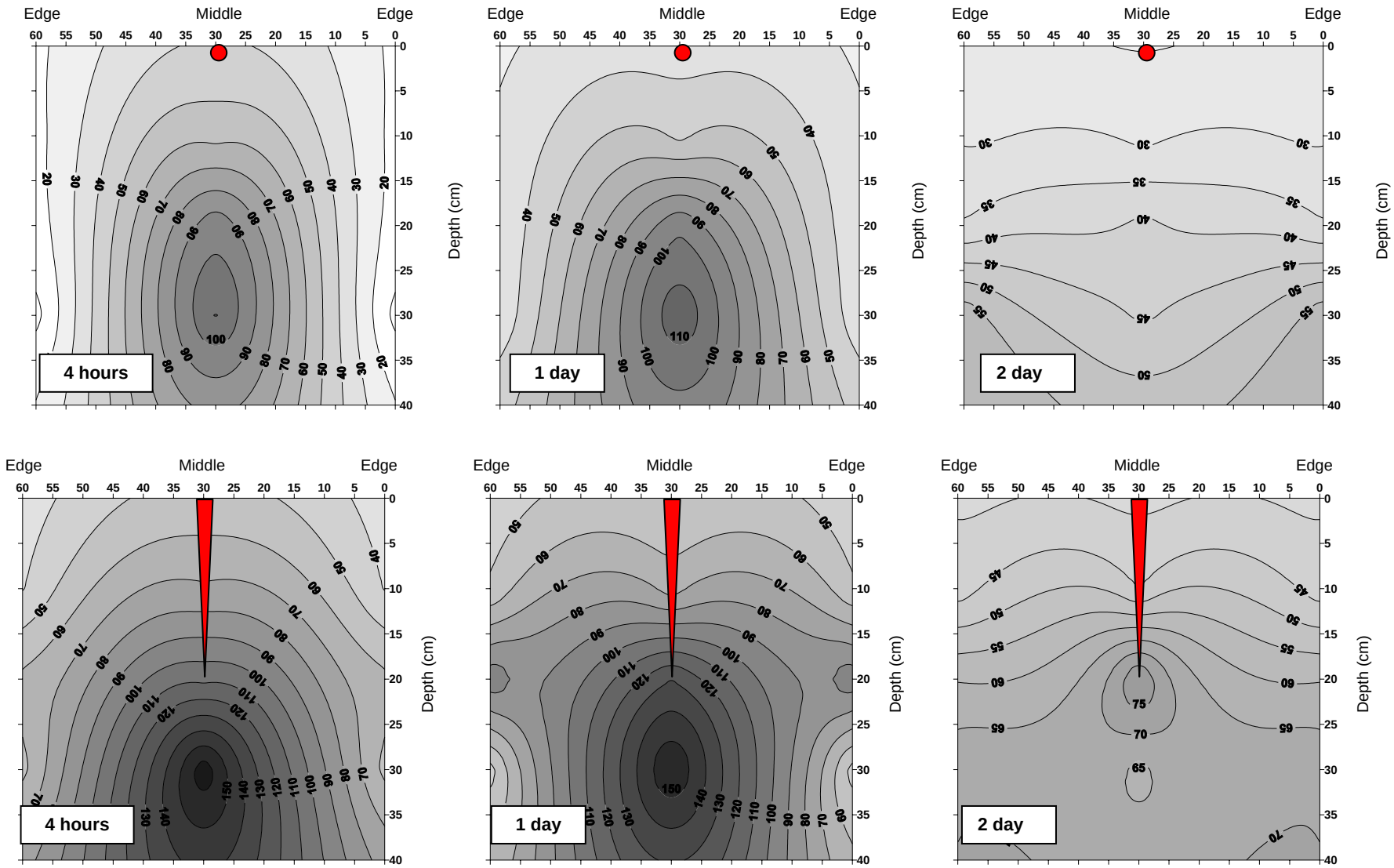


Figure 6.5. Contour plots, showing the lateral and vertical distribution of chloropicrin through strawberry beds, following drip fumigation (top) and shank fumigation (bottom) - 4 h, 1 day and 2 days after application (red circles represent drippers at the soil surface, red triangles represent shank position). Darker regions indicate higher fumigant gas concentration in the soil.

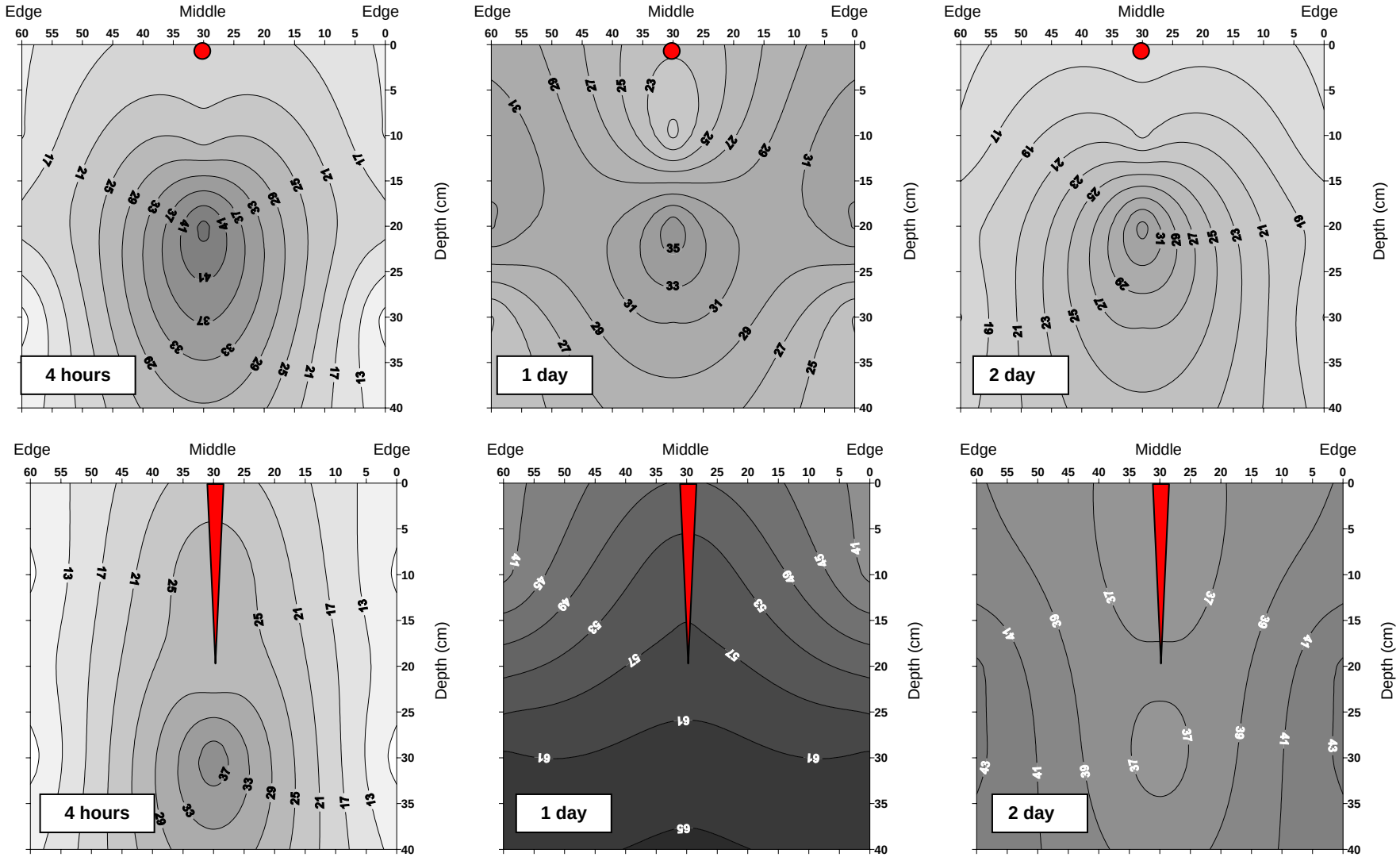


Figure 6.6. Contour plots, showing the lateral and vertical distribution of 1-3-D (Telone) through strawberry beds, following drip fumigation (top) and shank fumigation (bottom) 4 h, 1 day and 2 days after application (red circles represent drippers at the soil surface, red triangles represent shank position). Darker regions indicate higher fumigant gas concentration in the soil.

Plant growth parameters
Plants established, grew and cropped well in all fumigated plots, regardless of the fumigant or application method. Plant health, growth and subsequent fruit yield were substantially poorer in untreated plots (Figure 6.7).

Figure 6.7. Typical 'Camarosa' strawberry plants from unfumigated (top) and drip-applied chloropicrin-fumigated (bottom) plots, at the end of the growing season (February 2008).

A summary of fruit yield plus plant canopy and root health assessments is presented in Figure 6.8. Growth following each of the fumigant treatments was substantially greater than in unfumigated control plots. Fumigant application method (i.e. drip v. shank) did not have an obvious effect on subsequent plant growth for any of the three fumigants. On average, differences in plant health were relatively small between the various fumigation treatments, although root disease was slightly higher and canopy health poorer in the drip-applied TeloneC35 treatment. Root and canopy health were still substantially better in all fumigant treatments than in unfumigated control plots.

Strawberry fruit yield was equal or higher in drip-applied fumigant plots than in shank-applied plots of the same fumigants. Averaged across varieties, the highest fruit yields were in the drip-applied chloropicrin treatment. Fruit yield in all fumigation treatments was substantially higher than in the unfumigated controls.

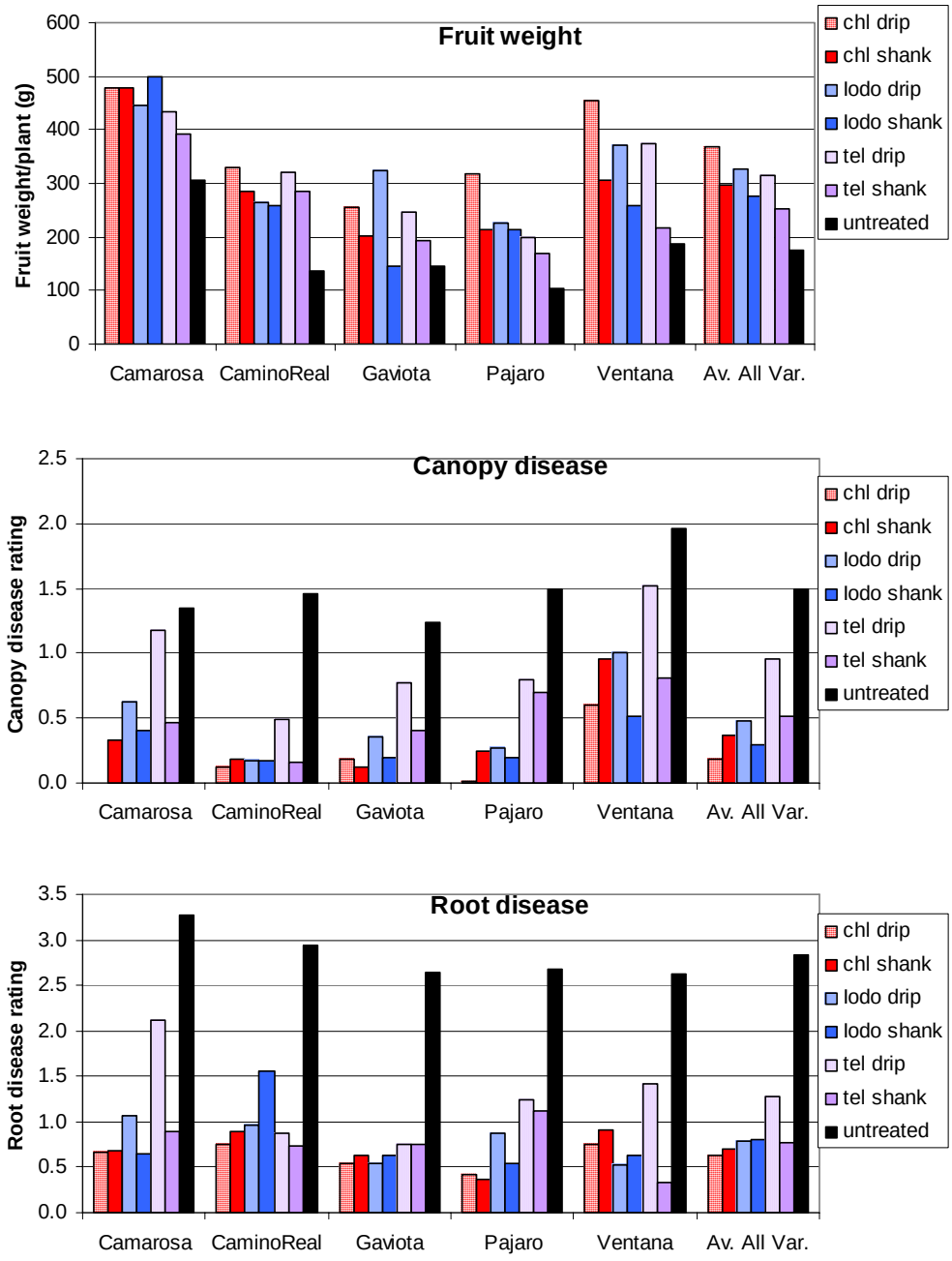


Figure 6.8. Fruit yield, canopy disease and root disease ratings in five strawberry varieties grown in plots fumigated pre-planting with chloropicrin, iodomethane or TeloneC35, applied via standard shank injection or through the drip irrigation system at Roselea in 2007. Total fruit is the average yield per plant, harvested from mid October 2007 until early January 2008. Canopy disease was scored on a 0 to 4 scale, where 0 was healthy and 4 was dead. Root disease was scored on a scale from 0 to 4 where 0 = all feeder roots healthy, 1 = 25% diseased, 2 = 50% diseased, 3 = 75% diseased, 4 = 100% diseased.

Roselea trial 2008/09

Pathogen and weed kill

Seed and *Phytophthora* survival data are presented in Table 6.4. The survival rate of both ryegrass seed and *Phytophthora* was high in bags at all three positions in untreated plots. By contrast, in beds fumigated via shank injection, regardless of the fumigant used, there was no survival of either ryegrass seed or *Phytophthora* propagules in bags in any of the three positions. The same was true for the iodomethane drip application treatment. However, with the other fumigants (chloropicrin, Telone C35, TeloneC60 and Fumasol) there was limited survival of ryegrass seeds, and very low survival of *Phytophthora* spores, in the bottom corner of beds. Mortality of both seeds and *Phytophthora* spores was high in the top and shoulder of beds in all drip treatments, except perhaps for the Telone C35 drip.

Table 6.4. Ryegrass seed germination and *Phytophthora cactorum* survival in bags buried at various positions in beds during fumigation by either drip or shank injection using a range of fumigants at Roselea in 2008.

Fumigation treatment	Ryegrass seed germination %			Phytophthora survival[a]		
	top	shoulder	bottom	top	shoulder	bottom
Chl -Drip	0	0	35	0	0	0
Chl -Drip-HIGH vol	0	0	15	0	0	1
Chl -Drip-LOW-vol	0	0	5	0	0	0
Chl –Drip pre-wet	0	0.5	25	0	0	1
Chl -Shank	0	0	0	0	0	0
Fum-Drip	0	0	75	0	0	0
Iodo-Drip	0	0	0	0	0	0
Iodo-Shank	0	0	0	0	0	0
TC35-Drip	0	30	30	0	1	1
TC35-Shank	0	0	0	0	0	0
TC60-Drip	0	0	22.5	0	0	0
TC60-Drip pre-wet	0	0	38.5	0	0	0
TC60-Shank	0	0	0	0	0	0
Untreated	95	95	100	3	4	4

[a] Phytophthora survival = number of bags out of four with live *Phytophthora* detected in baiting assays following bag retrieval.

Plant growth parameters

Plants established, grew and cropped well in all plots, regardless of the treatment or application method. A summary of fruit yield, canopy vigour, and plant health assessments is given in Table 6.5. There were no statistically significant differences in fruit production between treatments, possibly a reflection of the high variability between plots. However, fruit production was lowest in the untreated control beds, and there was a trend for fruit yield in drip-applied treatments to be slightly lower than in shank-applied treatments of the same fumigant.

Table 6.5. Fruit yield, plant vigour, and canopy disease scores averaged across five strawberry varieties grown in plots fumigated with chloropicrin, iodomethane, Telone®C35, Telone®C60 or Fumasol. Fumigants were applied pre-planting, via standard shank injection or through the drip irrigation system. Some drip-applied beds were pre-wetted or had fumigants applied in various water volumes (Table 2). Fruit yield is the average yield per plant, harvested from mid October until late December 2008. Plant Health was scored on a 0 to 4 scale, where 0 was a fully healthy canopy and 4 was dead. An asterisk * indicates where a given treatment mean, averaged across all varieties, is statistically significantly different (P<0.05) from the untreated control.

Treatment abbreviation	Fruit yield (g/m)	Canopy height (cm) Feb. 2009	Canopy Health Feb. 2009
Chl -Drip	2137	25.0*	0.40*
Chl-Drip-HIGH vol	2139	24.7*	0.51*
Chl-Drip-LOW vol	2031	21.4*	0.69*
Chl-Drip pre-wet	1897	24.1*	0.59*
Chl -Shank	2047	27.4*	0.20*
Fum-Drip	1890	22.1*	0.64*
Iodo-Drip	2071	21.8*	0.89*
Iodo-Shank	2172	27.5*	0.18*
TelC35-Drip	1787	19.1	1.43
TelC35-Shank	2164	26.4*	0.22*
TC60-Drip	1813	23.3*	0.65*
TC60-Drip pre-wet	1968	22.3*	0.98*
TC60-Shank	2148	27.4*	0.14*
Untreated	1768	17.9	1.55
P-value	*0.076*	*<0.001*	*<0.001*
LSD	*(347)*	*2.40*	*0.339*

By the end of the season, there were significant differences in plant growth and canopy health between treatments. Except for the TeloneC35 drip treatment, all fumigated treatments provided significantly better growth and canopy health than that in the untreated control. In general, across fumigants, there was a trend for plant height and canopy health to be significantly better in shank-fumigated than in drip-fumigated beds.

For the chloropicrin drip-applied treatments, the water volume used during fumigant application appeared to have an impact on plant performance. Differences in yield, plant height and health between the standard (7.5 L/m) and high (12.5 L/m) water rates were relatively small. But the low water rate (5 L/m) had, on average, lower fruit yield and canopy height, and poorer canopy health. This may result from poorer distribution of gas in the beds with the lower water volume, leaving large areas of the bed untreated.

Pre-wetting of beds a few days before drip application had minimal impact on any of the plant parameters measured, compared with similar treatments without pre-wetting.

Comparison of the various shank-injected fumigants (chloropicrin, Iodomethane, TeloneC35, TeloneC60), showed minimal differences for the various plant parameters

measured. Averaged across all varieties, fruit yield, plant height and disease symptoms were roughly equal among these four formulations.

Roselea trial 2009/10

Plant-back

Results for plant-back are presented in Tables 6.6 and 6.7. If planting occurred 1 or 2 weeks after fumigation, plants in all fumigation treatments had higher mortality than the untreated control. By week 3, mortality in chloropicrin shank and the two iodomethane treatments had dropped to rates close to that of the untreated controls. When planting after 4 weeks, mortality in the Fumasol drip treatments, and to a lesser extent the two TeloneC60 treatments, remained high. This indicates that with these products, the waiting time before planting should be longer than 4 weeks. All other products and applications were similar to the untreated controls after 4 weeks. Observations in the main plantings, made after 5 weeks, showed no obvious differences in early mortality between the various treatments.

Table 6.6. Percentage mortality of strawberry plants planted 1, 2, 3 or 4 weeks after soil treatment with various fumigants. Fumigation was in April and mortality data were recorded in September 2009.

Week	Chloro. Drip	Chloro. Shank	Fuma. Drip	Iodo. Drip	Iodo. Shank	TC60 Drip	TC60 Shank	Untreated
1	21.7	77.5	95.8	16.7	20.8	54.2	49.6	9.2
2	33.3	25.0	70.8	39.6	20.8	58.3	33.3	0
3	15.0	0	67.5	9.2	9.2	20.0	21.7	0
4	0	10.4	28.8	8.3	8.3	17.5	16.7	10.0

Table 6.7. Relative fruit yield from strawberry plants planted at weekly intervals following soil treatment with a range of fumigants. Fruit yield is expressed as a proportion of the yield from untreated controls planted at the same time. *Week 5 data are from main planting adjacent to the plant-back trial.

Week	Chloro. Drip	Chloro. Shank	Fuma. Drip	Iodo. Drip	Iodo. Shank	TC60 Drip	TC60 Shank	Untreated
1	0.968	0.074	0.005	1.252	1.295	0.394	0.236	1.0
2	0.835	0.766	0.294	0.812	0.962	0.328	0.765	1.0
3	0.926	1.574	0.204	0.958	1.195	0.691	0.915	1.0
4	1.736	1.484	0.881	1.711	1.478	1.157	1.058	1.0
5*	1.389	1.471	1.231	1.368	1.549	1.321	1.465	1.0

In Table 6.7, values less than 1.0 (or even marginally above 1.0) indicate potential problems with plant-back, as plant productivity was less than that in untreated controls planted at the same time. The fruit yield data reflected the mortality data, and showed that planting within 2 weeks of fumigation caused problems with all fumigants tested. Fumasol, and to a lesser extent Telone products, still showed plant-back problems after 4 weeks. Data from the main planting, 5 weeks after fumigation in the same block (described further below), indicate that by this time plant-back effects were probably minimal for all products, although it is possible with some treatments (e.g. Fumasol) that the positive growth/yield response to fumigation would have been even greater if the planting had been further delayed.

Soil gas readings using Gastec® tubes showed that soil concentrations of chloropicrin and iodomethane were negligible by week 3 (data not shown). Traces of Fumasol and Telone (~ 2 ppm) could still be detected after 4 weeks.

Pathogen and weed kill

Seed and *Phytophthora* survival data are presented in Table 6.8. The survival rate of both seed types and *Phytophthora* was high in bags in untreated plots. By contrast, in beds fumigated via shank injection, regardless of the fumigant used, there was no survival of either ryegrass seed or *Phytophthora* propagules in bags in either position. Similarly to the 2008 Roselea trial, when fumigants were applied via the drip irrigation system, there was limited survival of ryegrass seeds, and very low survival of *Phytophthora* spores, in the bottom corner of beds, even though mortality was high in the top/shoulder of beds.

Table 6.8. Ryegrass and clover seed germination and *Phytophthora cactorum* survival, in bags buried in various positions in strawberry beds during fumigation, by either drip or shank injection, with a range of fumigants at Roselea in 2009.

Fumigation treatment	Seed germination %				Phytophthora survival[a] (out of 4)	
	ryegrass		clover			
	shoulder	bottom	Shoulder	bottom	shoulder	bottom
Chl-Drip	0	29	0	28	0	0
Chl-Sh	0	0	0	0	0	0
Fum-Drip	0	63	0	54	0	0
MI-Drip	0	31	0	25	0	1
MI-Sh	0	0	0	0	0	0
TC60-Drp	0	0	0	0	0	1
TC60-Sh	0	0	0	0	0	0
Untr	100	100	100	100	100	100

[a] Phytophthora survival = number of bags out of four with live *Phytophthora* detected in baiting assays following bag retrieval.

Plant growth parameters

A summary of fruit yield, canopy vigour, and plant health assessments is given in Table 6.9. Except for the re-fumigated second year beds, fruit yield was significantly higher in fumigated than untreated plots, regardless of the fumigant or application method. There was a trend for fruit yield in drip-applied treatments to be slightly lower than in shank-applied treatments of the same fumigant, but these differences were not statistically significant.

At the end of the season, there were significant differences in plant growth and health between treatments. All fumigated treatments provided significantly better growth and canopy health than the untreated control. Similarly to fruit yield, there was a trend for shank-injected treatments to have better growth and canopy health than drip-applied

treatments. A comparison of results from the various shank-injected fumigants (chloropicrin, iodomethane, TC60) showed negligible treatment differences for plant height, health and fruit production. Averaged across all varieties, fruit yield, plant height and plant health were roughly equal across all treatments

Re-fumigation of the previous season's beds, via the existing drip fumigation system, was not successful. Although plant performance (health and yield), was slightly better than in comparable untreated beds, the yield from the re-fumigated beds was significantly less than that in newly formed and fumigated beds. This may be related, in part, to the soil structure in the beds. During planting of the second year beds, it was noted that the soil in those beds was very firm, making planting extremely difficult, as plant roots could not be pushed into the soil with the planting tool. When plants were dug up at the end of the season, it was noted that root growth in the replanted beds was poor, with minimal exploitation of the soil in the bed. This contrasted with first year beds, where root distribution within the beds was extensive. It was concluded that the silt-clay soil at Roselea is probably not suited to re-fumigation of beds.

Table 6.9. Fruit yield, plant vigour, and canopy disease score averaged across five strawberry varieties grown in plots fumigated with chloropicrin, Iodomethane (methyl iodide:chloropicrin 50:50), TeloneC60 or Fumasol (metam sodium), applied pre-planting via standard shank injection or through the drip irrigation system. Fruit yield is the average yield per plant harvested from mid October until late December 2009. Canopy health was scored on a 0 to 4 scale, where 0 was healthy and 4 was dead.

Treatment abbreviation	Fruit yield (g/pl)	Canopy height (cm) Feb. 2009	Canopy health Feb. 2009
Chl -Drip	351.1*	21.8*	1.33*
Chl -Shank	371.9*	24.1*	0.79*
Fum-Drip	311.2	20.4*	1.61*
Iodo-Drip	345.7*	21.5*	1.61*
Iodo-Shank	391.4*	24.6*	0.78*
TC60-Drip	333.7*	20.2*	1.68*
TC60-Shank	370.2*	25.1*	0.73*
Untreated	252.7	16.9	2.26*
P-value	<0.001	<0.001	<0.001
LSD	69.9	2.09	0.409

An asterisk * indicates where a given treatment mean, averaged across all varieties, is significantly different ($P<0.05$) from the untreated control.

6.3.2 Australian strawberry fruit trial

Millgrove trial 2008/09

Fumigant Residues

Concentrations of methyl iodide in strawberry beds were approximately 600 ppm immediately after fumigation in both the shank injected and drip fumigated applications (Figure 6.9). However, by 7 days after fumigation concentrations of methyl iodide had reduced by approximately 75 %. At 14 and 21 days after fumigation concentrations of methyl iodide were almost undetectable, depending on the application. As such, results indicate that methyl iodide residues remained in strawberry beds for around 21 days, under the conditions of this trial.

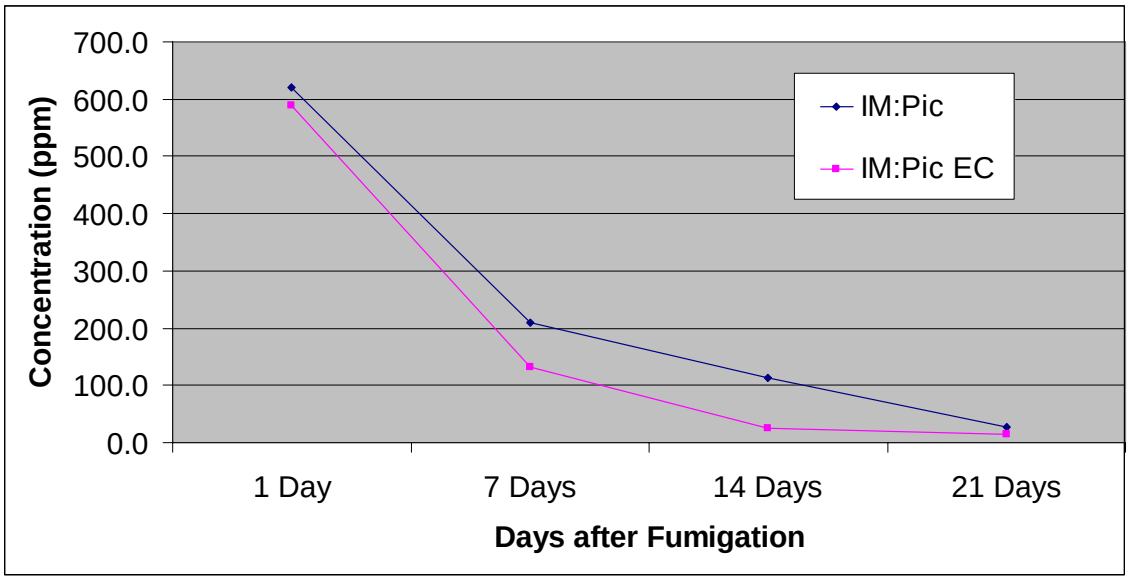


Figure 6.9 - Concentration of methyl iodide (IM, ppm) in soil following application via drip fumigation and shank injection (Not significant, $p > 0.05$).

Vegetative growth

Early vegetative growth (leaf number and leaf area, 91 days after fumigation) of strawberry plants was similar in all fumigant treatments (Figures 6.10 and 6.11). The vegetative growth data indicated that strawberries grown in MB, IM and Pic treated soil enhanced plant growth as they had significantly higher leaf area, compared to plants grown in untreated soil. As such, the vegetative growth data gave no evidence to suggest that IM negatively affected strawberry fruit growth (eg phytotoxicity), providing sufficient time is allocated between fumigation and planting (ie plant-back time).

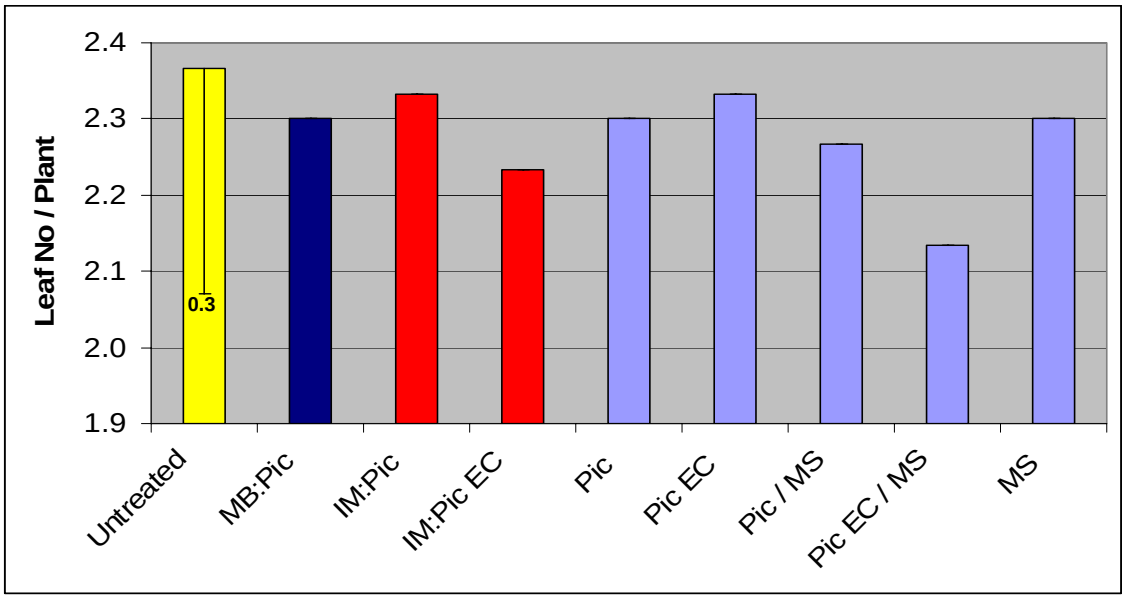


Figure 6.10 – Average leaf number on strawberry plants grown in soils treated with various fumigants, 91 days after fumigation. Bar represents Least Significant Difference values for the average leaf number, where p = 0.05

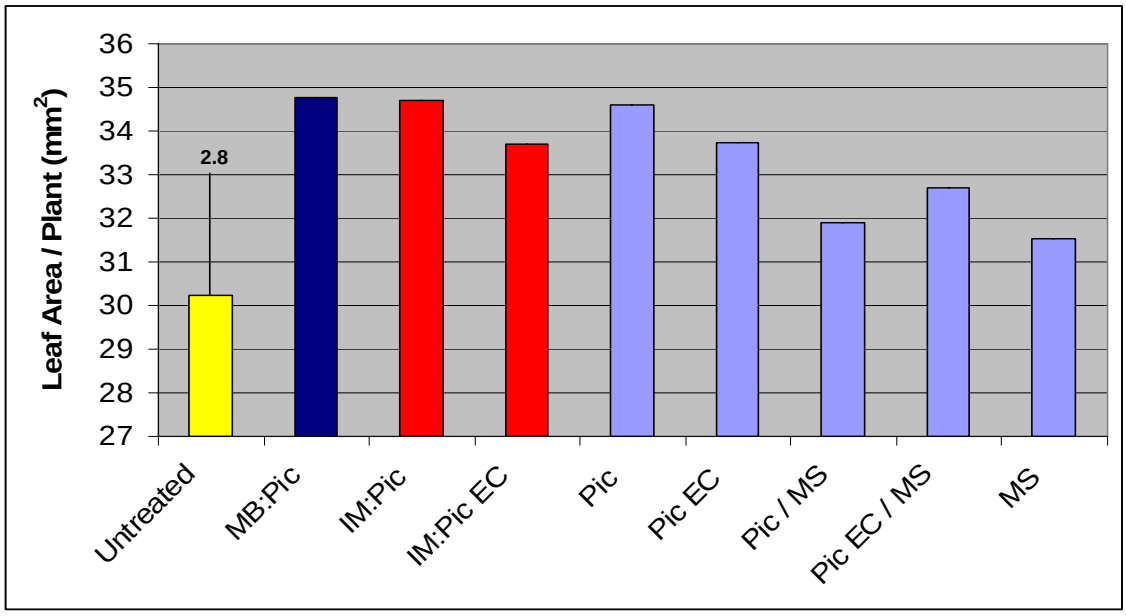


Figure 6.11 – Average leaf area (mm^2) of strawberry plants grown in soils treated with various fumigants, 91 days after fumigation. Bar represents Least Significant Difference values for the average leaf area, where p = 0.05.

Efficacy (weeds)

The dominant weed species emerging across the trial site was *Poa annua* (winter grass). All treatments reduced numbers of *Poa annua* except for Pic. In addition, all treatments significantly reduced the total number of weeds by over 75 %, in comparison to the untreated control, except for Pic (Figure 6.12). No treatment was effective at reducing numbers of *Trifolium repens* (white clover).

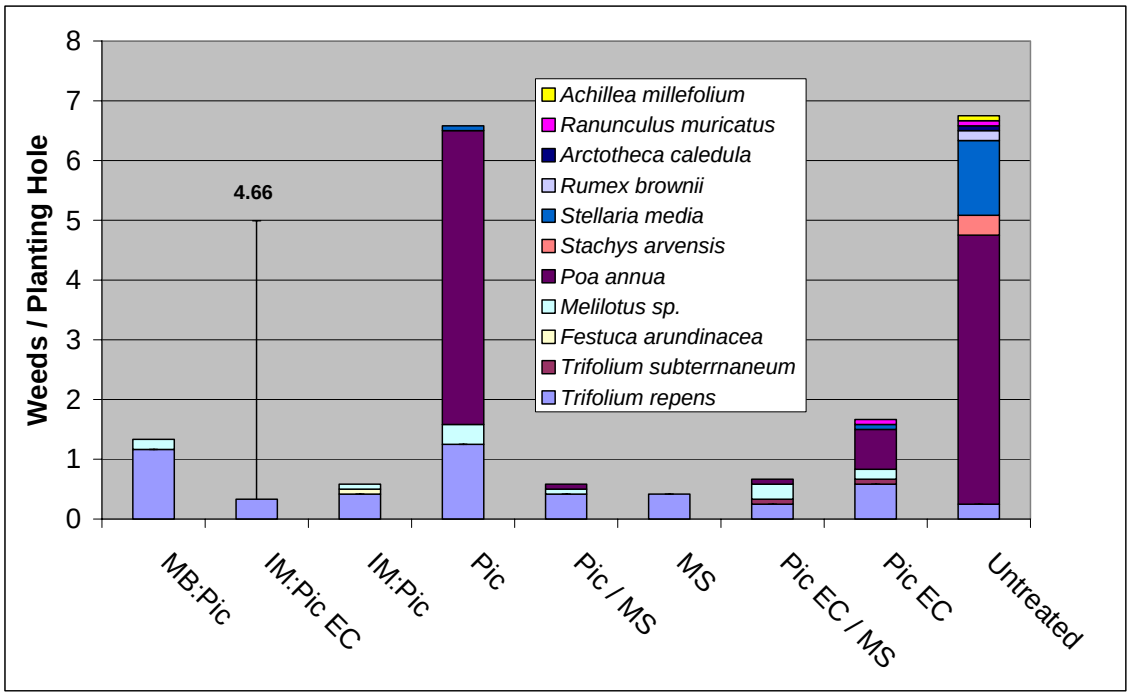


Figure 6.12 - Effect of fumigation on weed populations, 91 days after fumigation. Bar represents Least Significant Difference values for the total weed numbers, where p = 0.05.

Yield

There was no significant difference in marketable fruit yields between treatments (Figure 6.13). This data represents early fruit production when plants were still establishing (first 2 months of fruit production) and yields are normally at their lowest.

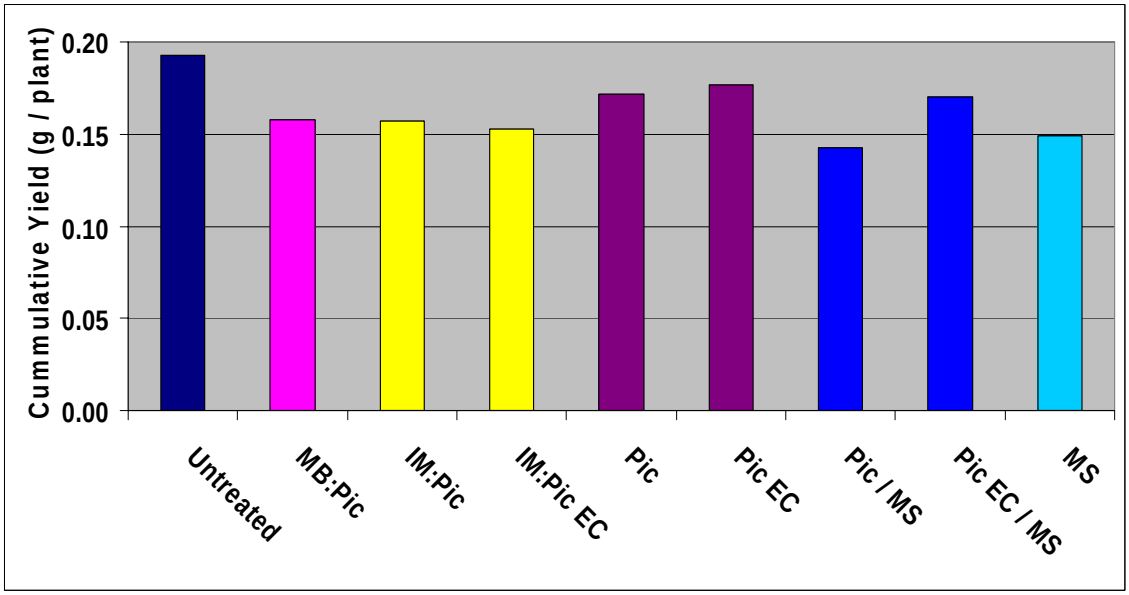


Figure 6.13 – Effect of fumigation on marketable fruit yields kg/plant from November – December, 08. Data was not significant, where p = 0.05.

6.3.3 NZ Strawberry runner trial

Katikati trial, 2008

Buried pathogen and weed kill

Viable *Phytophthora* was retrieved from all samples taken from untreated control plots, but could not be retrieved from any fumigated plots, regardless of the fumigant, rate or plastic used, or whether the bag was buried at 5 or 20 cm depth.

Germination of ryegrass seed was close to 100% in bags buried in untreated control plots, but no seed germinated in bags buried in any of the fumigated plots. Clover seed germination was approximately 60% in bags from untreated plots, and 0% in 60 out of 64 bags buried in fumigated plots. The remaining four bags had between 2 and 4% clover germination, with no pattern relating to fumigant, rate or film.

Weed emergence

Untreated control plots had substantially more weeds than any of the fumigated plots (Figures 6.14). There were no significant differences in weed growth between the various fumigant, rate and plastic mulch combinations.

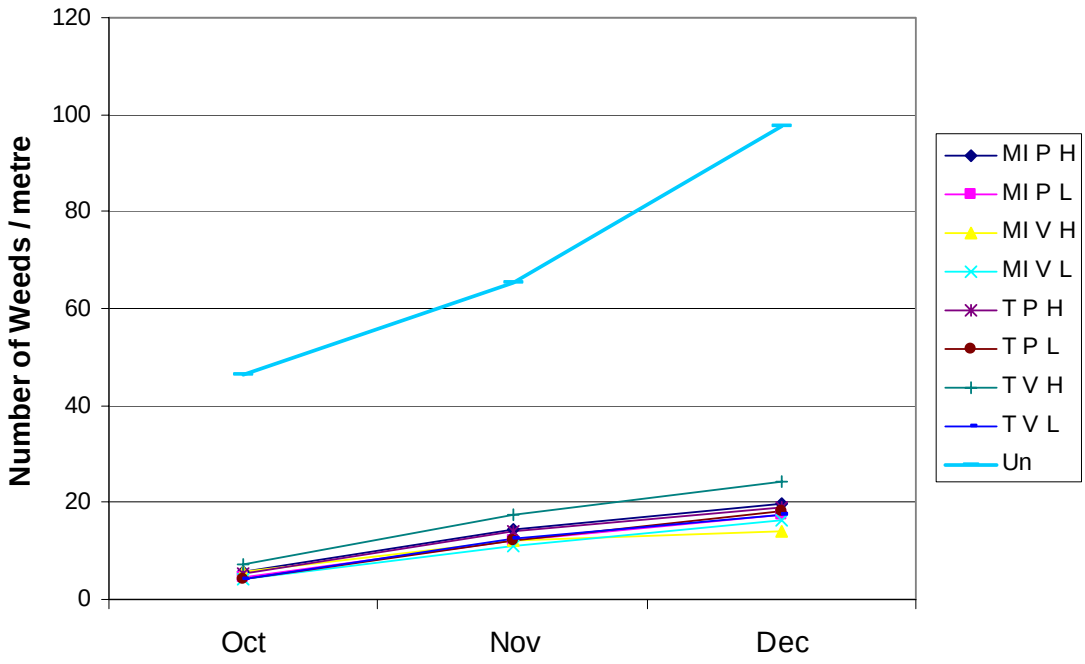


Figure 6.14. Cumulative weed counts in strawberry runner bed plots treated with various fumigant (MI=methyl iodide, T=TeloneC35, Un=untreated) and rates (H=high, L=low) and covered with either VIF (V) or standard polyethylene (P) film.

Runner growth and yields

Vegetative plant yield, measured in terms of total biomass production, number of marketable plants or weight of marketable plants, was significantly higher in all the fumigant treatments than in the untreated controls (Table 6.10; Figure 6.15). Few of the differences in plant productivity among the various fumigant, rate or mulch combinations were statistically significant. The low rate of TeloneC60 with polyethylene cover tended to have the lowest productivity of all the fumigant treatments.

Plant and root health was good in all fumigant treatments, but noticeably poorer in untreated control plants, with high levels of feeder root decay in the latter.

Table 6.10. Plant growth assessments of strawberry runner plants grown in beds treated with TeloneC60 or Methyl iodide (at high or low rates), or left untreated. Plots were covered with either VIF mulch or standard polyethylene. Data are counts/weights per mother plant.

Treatment	February assessment			May assessment		
	Mother Weight (g)	Daughter weight (g)	Daughter number	Marketable Plant Weight (kg)	Bulk Weight (kg)	Marketable Plant number
MI P H	330*	748*	16.4*	8.14*	8.76*	242*
MI P L	344*	764*	15.1	9.64*	10.30*	263*
MI V H	334*	704*	16.2*	8.57*	9.09*	260*
MI V L	309	752*	15.8	7.87*	8.40*	233*
T P H	376*	736*	16.5*	8.06*	8.65*	248*
T P L	334*	828*	18.8*	7.11*	7.62*	213*
T V H	314	805*	18.7*	7.63*	8.13*	233*
T V L	337*	700*	15.3	8.41*	8.95*	253*
Untreat	263	466	12.4	4.16*	4.36	116
P -value	*.011*	*<0.001*	*0.065*	*<0.001*	*<0.001*	*<0.001*
LSD	*57*	*142*	*(4.072)*	*1.89*	*1.99*	*53*

* An asterisk* indicates that means were significantly different from those in the untreated control for the same parameter on the same site.

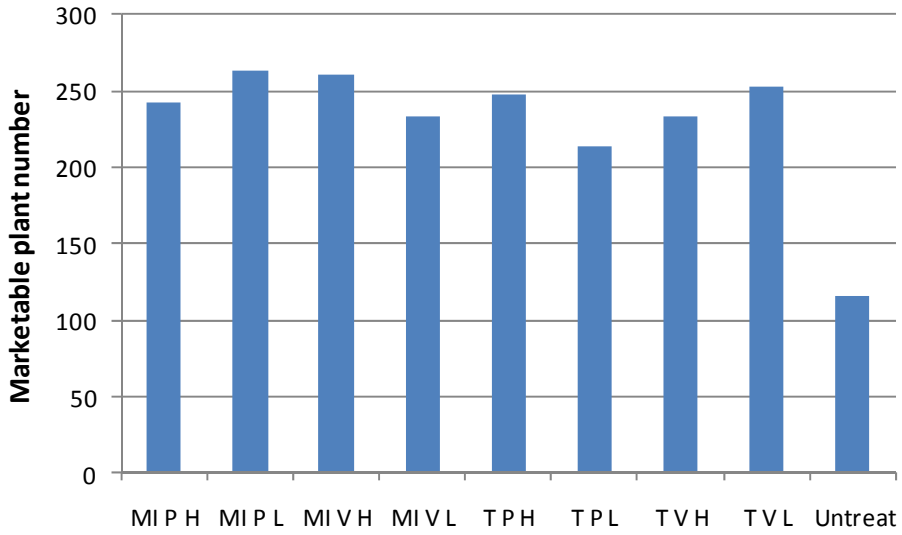


Figure 6.17. Average number of marketable strawberry runner plants produced in runner bed plots treated with various fumigants (MI=methyl iodide, T=TeloneC35, Untreat=Untreated), rates (H=high, L=low) and covered with either VIF (V) or standard polyethylene (P) film.

Fruit yield of runners

Plant growth, health, and fruit yield data are presented in Table 6.11. There were no significant differences in growth, health or yield of plants sourced from the various

fumigant, rate, or plastic film treatments in the runner beds. Average fruit yield and canopy health was poorer in plants that came from untreated runner plots than from any of the fumigated plots, but differences were not statistically significant.

Table 6.11. Strawberry plant growth, health and fruit production in fruiting beds, following transfer from nursery runner beds treated with various fumigant/plastic/rate combinations.

Treatment	Fruit yield (g/plant)	Plant height	Canopy health
MI P H	581	32.4	0.59
MI P L	599	31.9	0.44
MI V H	579	32.4	0.13
MI V L	666	33.4	0.33
T P H	626	33.3	0.58
T P L	549	30.7	0.62
T V H	610	30.1	0.65
T V L	579	30.4	0.38
Un	454	30.5	0.97
P-value	0.191	0.730	0.471
LSD	(170)	(4.67)	(0.797)

6.3.4 Australian strawberry runner trial

Toolangi trial, 2008/09

All fumigant/film treatments increased runner yields above the untreated control, except low rate MB:Pic under VIF. There was no difference in yield between MB:Pic and MI:Pic treatments or between VIF and LDPE treatments (Figure 6.17).

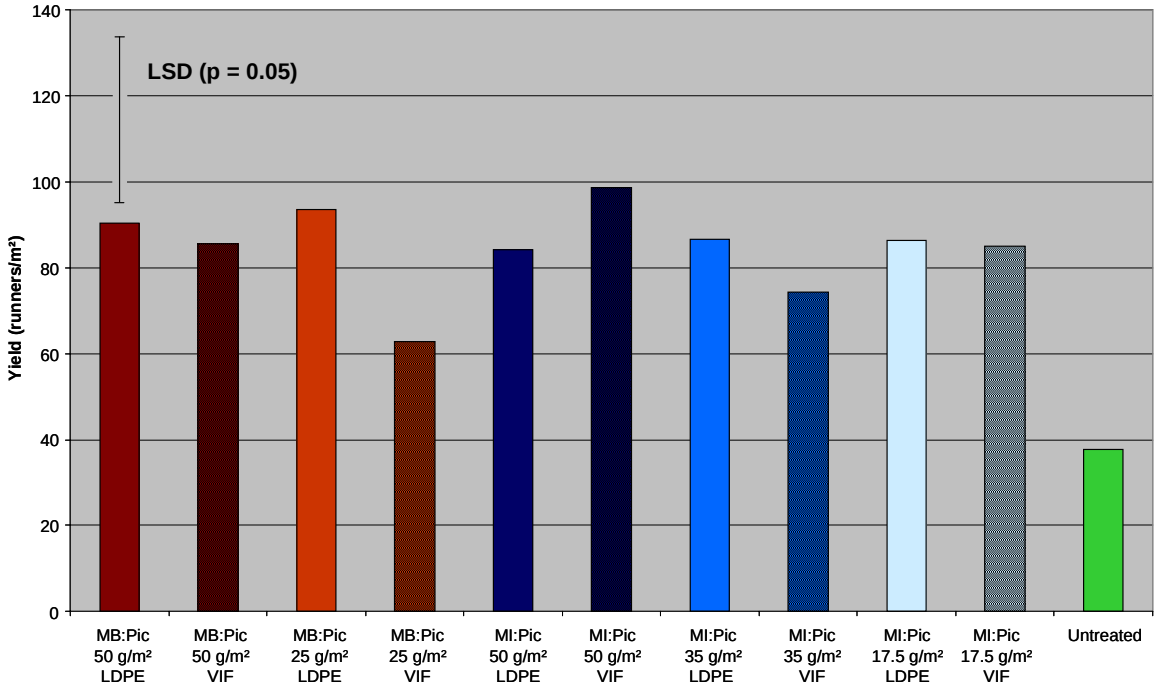


Figure 6.17. Effect of fumigants and films on strawberry runner yields from Gaviota grown at Toolangi. The bar is the least significant difference where p = 0.05.

6.4 Discussion

Overall the alternative fumigants chloropicrin, Telone® products, metam sodium and methyl iodide:chloropicrin showed strong potential for soil disinfestation in the strawberry industries. In particular, methyl iodide:chloropicrin showed the potential to control weeds and pathogens, and produce strawberry runner and fruit yields to equivalent levels as methyl bromide:chloropicrin, irrespective of application method (drip or shank) or use of barrier films (see results from Chapters 4, 5, and 6). Moreover, methyl iodide consistently showed short residence times in soil compared with other alternative fumigants. This correlated to shorter plant-back times for methyl iodide:chloropicrin in strawberries, and reduced risk of crop phytotoxicity. The combination of metam sodium followed by chloropicrin also showed promise in one fruit trial in Australia, but growers must note that these products are highly reactive when mixed. This means that they must be applied in separate operations.

6.4.1 Drip fumigation trials

The various 'drip v. shank application' trials carried out in NZ and Australia gave conflicting results. In the first trial (2007) at Roselea in NZ, all fumigants tested (iodomethane, chloropicrin and TeloneC35) produced greater fruit yields in drip-applied than shank-applied plots. The following year, fruit yields were not significantly different between the two application techniques, although tending to have slightly higher yields following shank application. Plant growth and health was also superior following shank application. In the 2009 trial at Roselea there was superior growth, health and fruit yield in shank-applied beds than in drip-applied beds. At the Millgrove trial in Australia, drip application of chloropicrin markedly increased weed control compared with shank application. However, there were no differences in yields between drip- and shank-applied treatments in the trial

Studies of gas movement within the soil indicate good dispersal of fumigants throughout the beds, whether or not application was via drip or shank injection. For both chloropicrin and methyl iodide, patterns of dispersal after 24 h looked very similar for both application systems, although dispersal of Telone® (1,3 dichloropropene) appeared slightly slower following drip application.

The studies with buried bags are very useful, as they reflect the distribution of fumigant gases in concentrations that are biologically important. Similar results were obtained with both weed seed and *Phytophthora cactorum* assays, i.e. that pathogen and weed kill was very good in the centre and shoulder of beds with both application techniques. However, the bottom outside corner of beds was, on occasion, a reservoir for pathogen or weed survival, particularly following drip application. This indicates that lethal concentrations of the fumigant gases sometimes failed to reach these distal portions of the bed. The biological significance of these potential reservoirs is unclear. In a crop that is replaced annually, it is likely that by the time roots penetrate this region, the pathogen may have minimal impact.

There were some practical problems with drip application in some trials. There was a noticeable 'settling' or collapsing of some beds in at least two of the trials. These problems could possibly be avoided either by slower application rates or by pulsing the application (e.g. six 20-minute bursts, rather than one 2-hour application).

Growers may also need to consider slope on their farms and the use of pressure-compensated tapes with drip fumigation.

Drip application of fumigants is potentially a viable option for Australian and New Zealand strawberry fruit growers. But before adopting this system on large farms, it is recommended that growers test the technique on small areas first, so that its effectiveness can be determined, and potential problems on each individual soil type resolved. The advantages of drip application (worker safety, independence from weather and soil conditions during application, improved industry-wide logistics during the fumigation season), may be worth the effort.

6.4.2 Barrier film trials

Barrier films (VIF) are less permeable to gaseous emissions of fumigants than standard films (LDPE). For this reason, the use of barrier films may allow lower application rates of some fumigants. In the current trials, however, there was no evidence of barrier films improving the efficacy of fumigants against weeds and pathogens, or allowing reduced rates of fumigants. This is in contrast to many studies overseas, but is consistent with our previous trials on barrier films in Australia. The reason for this is that emission rates of fumigants in Toolangi soils in Australia are low compared with soils in the US runner industry due to high clay content and high organic matter. For example, only 6% of applied methyl iodide is emitted from Toolangi soils over a 10 day period through standard film and bare soil (see Chapter 7). Therefore, use of barrier films does not retain much fumigant (less than 6% of the application rate), especially when the film remains in place for short periods, such as in the runner industry.

7. Environmental and Bystander Safety of Methyl Bromide Alternatives (Activity 5)

7.1 Summary

An air monitoring study was conducted following an application of 50:50 methyl iodide:chloropicrin in Toolangi, Victoria, which is in the largest strawberry growing area in Australia. The application rate was 500 kg/ha and a standard low density polyethylene (LDPE) tarp was used. Air monitoring stations were placed at a range of 10-600 meters from the edge of the field. The air monitoring data were used to estimate the flux of methyl iodide for 24 different periods across 10 days after the application. Chloropicrin was not detected at any of the off-field monitors (but was detected at a monitoring site on the field); thus, fluxes could not be estimated for chloropicrin.

The estimated flux for methyl iodide ranged from about 5 $\mu g/m^2$/sec immediately after the application to 7.5 $\mu g/m^2$/sec after the tarp was punched and removed, to about 0.25 $\mu g/m^2$/sec at the end of the 10-day monitoring period. Over the first 24 hours, only about 1.5% of the applied methyl iodide mass was emitted. Over the 10-day monitoring period, about 6% of the applied methyl iodide mass was emitted.

The flux estimates at Toolangi were substantially lower than for broadcast applications in the United States, where between 35-51% of the mass was emitted over the first 24 hours. One likely reason for the difference was that the soils in the U.S. were sands or sandy loams, while the soil at Toolangi was clay, which would impede the diffusion of gas through the soil profile. The flux estimates using the air monitoring were consistent with other measurements made with a flux chamber.

The concentrations at the offsite monitors were also substantially less than the U.S. Environmental Protection Agency (EPA) reference concentration of 870 $\mu g/m^3$. The maximum concentration 10 meters from the field edge was 199 $\mu g/m^3$ across 207 measurements. Consistent with this observation, the PERFUM2 model predicted that buffer zones were not necessary when assuming the fluxes from Toolangi with a 5-year meteorological dataset from Coldstream in Victoria.

This study was the key work required by the Australian Pesticides and Veterinary Medicines Authority (APVMA) to allow a decision on registration of methyl iodide and for review of the registration status of chloropicrin.

7.2 Background

This bystander safety study was designed in collaboration with the Office of Chemical Safety and Environmental Health (OCSEH), the Department of Sustainability, Environment, Water, Population and Communities (DSEWPC), the Australian Pesticides and Veterinary Medicines Authority (APVMA) and Dr Rick Reiss of Exponent, USA who ensured that the design would enable comparisons with the USA studies.

Key features requested by OCSEH and DSEWPC were:

- It should be conducted in a typical soil type, and topography and climate (ie Toolangi)
- Fumigation should be done at the maximum application rate using typical tyne injection equipment
- The barrier film should be low density polyethylene, which is current standard practice in Australia, not the newer virtually impermeable films which were used in some of the USA studies.
- Air sampling should be done at a nose height of 1.8 m (OCSEH) and at 15 cm (DSEWPC)

All of these requirements were met and are specifically indicated in this chapter.

This chapter analyses air monitoring data to estimate fluxes, compares the air monitoring results to the U.S. Environmental Protection Agency (EPA) toxicity levels of methyl iodide and chloropicrin, and provides an analysis using the standard U.S. risk assessment model for bystander exposure for fumigants, PERFUM.

7.3 Materials and methods

7.3.1 Trial site

The trial site was located at Toolangi, Vic (37º31'26.78''S 145º30'00.79''E), where 80% of Australia's strawberry transplants are grown (Fig 7.1). The soil type was a Ferrosol (clay texture). The prevailing wind at the site is N – NW. There was a 10º slope across the trial site, running from east to west.

On 20-Oct-10, a 65 × 65 m block was fumigated with a mixture of methyl iodide: chloropicrin (50:50), supplied by Arysta LifeSciences, applied at a target rate of 500 kg/ha by a commercial fumigator (R&R Fumigation Services, Bayswater).

The product was applied with a commercial fumigation rig (Fig 7.2) equipped with a calibrated flow meter, through 12 tynes (20 cm deep) spaced 20 cm apart.

Simultaneously, the soil was sealed with low-density polyethylene (35 µm thick).

The mass of the fumigant cylinder was weighed prior to and after fumigation to verify application rates of the product.

On 25-Oct-10 at 09.30 (5 days after fumigation), the LDPE seal covering the treated soil was cut and removed (Fig 7.3). This is consistent with the average time that strawberry runner growers can leave the LDPE seal on fumigated soil (due to wind and wet weather).

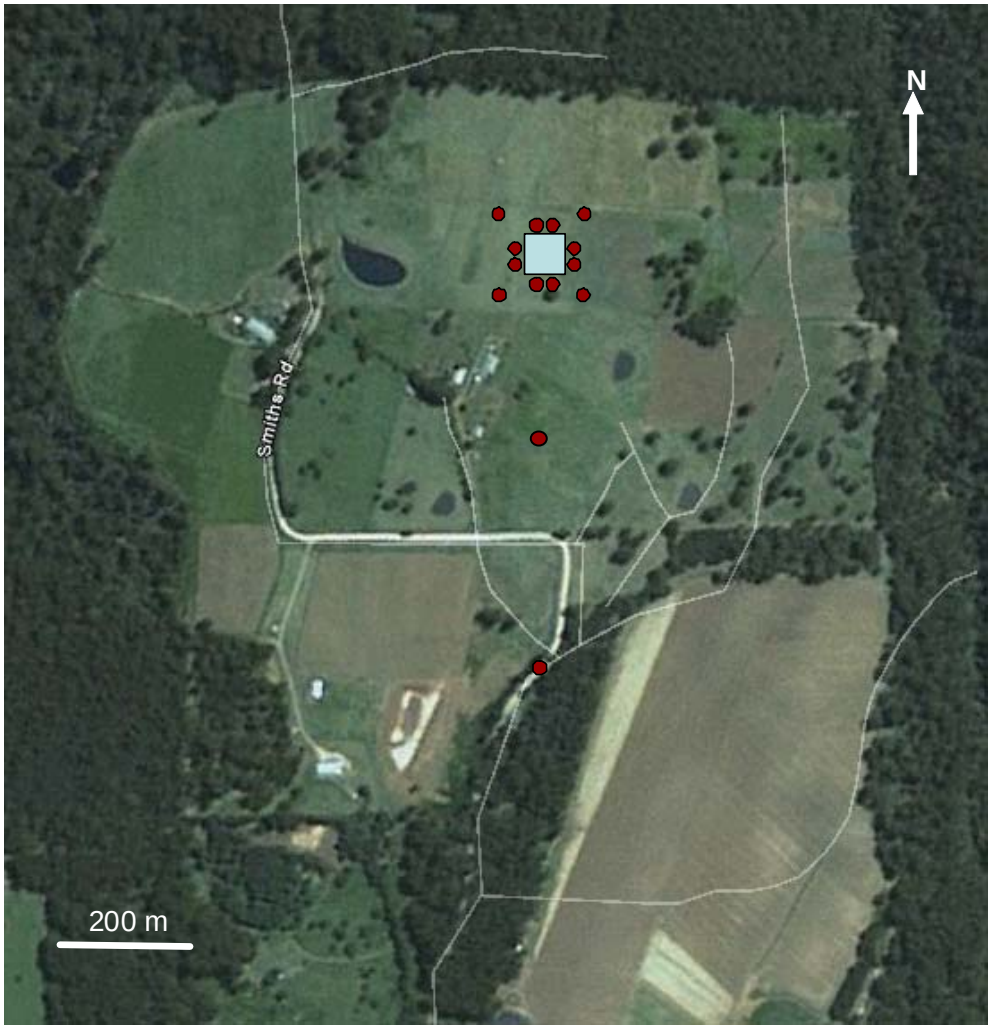


Figure 7.1. Location of the trial site in Toolangi Victoria. The blue square represents the treated field, and the red dots mark the location of air monitoring stations.

7.3.2 Air monitoring

A weather station was set up on the trial site to datalog wind speed, wind direction, and air and soil temperature every 10 min.

Thirty SKC air pumps were mounted on steel posts, 1.8 m above ground (as per OCSEH's request), around the treated block (Table 7.1, Figure 7.4). Six pumps (measuring stations 1, 2 and 11) were set up at 15 cm above ground (as per DSEWPC's request). There were two pumps at every measuring station (one to measure chloropicrin on XAD-4 tubes, and one to measure methyl iodide on activated charcoal tubes), making a total of 30 pumps. Pumps were encased in plastic boxes to protect from them from rain.

Figure 7.2. Fumigation of the trial site on 20-Oct-10.

Figure 7.3. Removal of LDPE barrier film on 25-Oct-10.

Commencing at 2-hours after fumigation, air samples were taken every 6 hours for the first 2 days after fumigation, and then every 12 hours until day 10 of the trial (see Table 7.2). Prior to measurement, pumps were calibrated with a DryCal flow meter to produce a flow rate of approximately 50 ml/min. The actual flow rate was recorded for every pump. The appropriate sorbent tube was attached to the pump and air sampled through the tubes for a period of approximately 2 hours (the actual sample time for each tube was recorded). Following sampling, the sorbent tubes were

Table 7.1. Description and rationale for the air monitoring stations used in the trial.

Station No.	GPS Location	Height* (cm)	Distance† (m)	Direction†	Rationale
1	S 37º31.454' E 145º30.042'	15	0	-	Set up 15 cm above the fumigation plot to measure the maximum concentration of fumigant emissions as per DSEWPC's request.
2	S 37º31.460' E 145º30.049'	15	10	S	Set up 10 m from the fumigation site and are most critical stations for fumigant dispersion modelling. Downwind stations 2 and 3 were set up at heights of 15 cm and 180 cm, respectively, as per DSEWPC's request.
3	S 37º31.460' E 145º30.049'	180	10	S	
4	S 37º31.460' E 145º30.031'	180	10	S	
5	S 37º31.444' E 145º30.012'	180	10	W	
6	S 37º31.432' E 145º30.014'	180	10	W	
7	S 37º31.416' E 145º30.037'	180	10	N	
8	S 37º31.416' E 145º30.054'	180	10	N	
9	S 37º31.432' E 145º30.073'	180	10	E	
10	S 37º31.444' E 145º30.070'	180	10	E	
11	S 37º31.475' E 145º30.080'	15	45	SE	Set up at 45 m, downwind, from the fumigation site. Stations 11 and 12 were set up at heights of 15 cm and 180 cm, respectively, as per DSEWPC's request.
12	S 37º31.475' E 145º30.080'	180	45	SE	
13	S 37º31.472' E 145º29.993'	180	45	SW	
14	S 37º31.562' E 145º30.021'	180	200	S	Set up at 200 m from the fumigated site as an intermediate distance between 45 m and 600 m (the anticipated limit of detection) as requested by OCSEH
15	S 37º31.821' E 145º29.966'	180	600	S	Set up at the anticipated limit of detection as requested by OCSEH

* above ground, † from the fumigated field

retrieved, capped, and placed on dry ice for later storage at 0ºC. Flow rates of each pump were again measured and recorded. Rainfall was recorded at each air sampling time.

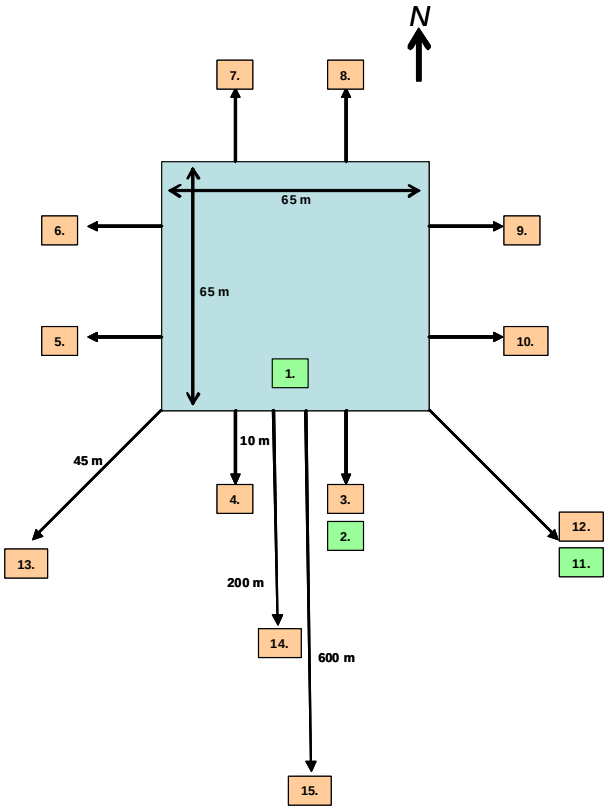


Figure 7.4. Locations of air monitoring stations. (Green sites were set at a height of 15 cm and orange sites at 1.8 metres).

7.3.3 Fumigant analysis

In the laboratory, fumigant residues were extracted from sorbent tubes using 1 mL of carbon disulphide or ethyl acetate, respectively, for carbon and XAD. Extract (2µL) was injected in split mode using an auto-sampler (CTC Analytics CombiPal) onto a 60m × 0.25mm ID DB-VRX 1.4µm column (J&W) that was temperature programmed from 50˚C to 100˚C at 10˚C/min., held for 8min., then temperature programmed to 200˚C at 20˚C/min.

Gas chromatography was performed using a gas chromatograph (Varian 3800) with the injector temperature set to 130˚C, split ratio set to 1:25 and the helium carrier gas flow set to a constant 1.2mL/min.

Selected ion monitoring was performed using a mass spectrometer (Varian 1200L) in electron impact ionisation mode. Methyl iodide (retention time ~ 5.6min.) was detected by monitoring each of m/z127, m/z141, and m/z142 with a 160msec dwelltime. Chloropicrin (retention time ~ 12.7min.) was detected by monitoring each of m/z117, m/z119, and m/z121 with a 160msec dwelltime.

The concentration of methyl iodide and chloropicrin in the air was determined from the time over which the air was sampled, the average flow rate over the sampling time, and the concentration of two compounds recovered.

Table 7.2. Sampling times following fumigation and after the removal of LDPE barrier film at each station in the trial.

Sample No.	Date	Time	Hours after Fumigation	Hours after Removal of Barrier Film
1	20-Oct-10	16:00	2	-114
2	20-Oct-10	22:00	8	-108
3	21-Oct-10	04:00	14	-102
4	21-Oct-10	10:00	20	-96
5	21-Oct-10	16:00	26	-90
6	21-Oct-10	22:00	32	-84
7	22-Oct-10	04:00	38	-78
8	22-Oct-10	10:00	44	-72
9	22-Oct-10	22:00	56	-60
10	23-Oct-10	10:00	68	-48
11	23-Oct-10	22:00	80	-36
12	24-Oct-10	10:00	92	-24
13	24-Oct-10	22:00	104	-12
14	25-Oct-10	10:00	116	0
15	25-Oct-10	22:00	128	12
16	26-Oct-10	10:00	140	24
17	26-Oct-10	22:00	152	36
18	27-Oct-10	10:00	164	48
19	27-Oct-10	22:00	176	60
20	28-Oct-10	10:00	188	72
21	28-Oct-10	22:00	200	84
22	29-Oct-10	10:00	212	96
23	29-Oct-10	22:00	224	108
24	30-Oct-10	10:00	236	120

7.3.4 Indirect flux estimation method

The California Department of Pesticide Regulation (CPDR) has developed a modelling methodology to estimate the flux of a fumigant from a field following application based on measurements of the concentration of the fumigant around the field (Johnson et al., 2010).

The CDPR methodology employs a standard air dispersion model developed by the U.S. Environmental Protection Agency (EPA) called the Industrial Source Complex (ISC) model (EPA, 1995). This analysis uses version 3 of the short-term part of ISC, which is called ISCST3. The model assumes that the dispersion of a plume downwind of a release can be estimated as a Gaussian distribution in both the vertical and lateral directions, with the amount of dispersion dependent on the stability of the atmosphere, the wind speed, and the distance from the source. ISC provides predictions of the concentration of an airborne compound downwind following a release, and requires the following input data:

- The flux of the compound (i.e., the amount of mass of the compound that is being emitted from the field per unit time for a given area), and;

- The geographical dimensions of the field for an area source such as the emission of a fumigant from a field following an application, ; and,
- The meteorological conditions during the modelling period, including wind speed, wind direction, and atmospheric stability.

In the current study, the downwind concentrations of fumigants were measured (see above), and the objective was to determine the flux of the compounds. Therefore, the ISC model was used to "back-calculate" the flux.

The goal of the back-calculation is to determine the flux that best explains (statistically) the observed measurements. The ISC model was run using a nominal flux (chosen, in this case, to be 100 $\mu g/m^2/sec$), and programmed to estimate the concentration at each of the measurement locations. Even if the nominal flux does not end up being the correct flux, it is not necessary to run the model again because the flux and the predicted concentrations are exactly proportional (e.g., a doubling of the flux results in a doubling of the downwind concentration). Therefore, given the results of a single model run, the concentrations at the air monitoring stations could be determined for any flux by multiplying the nominal concentration by the ratio of the flux and the nominal flux. The predicted concentrations at the measurement locations are statistically compared with the measured values to estimate the actual flux.

The fluxes are estimated using a linear regression of the measured concentration (y-intercept) and modelled concentration (x-intercept). The regression is constrained through zero because there needs to be a 1:1 relationship between the nominal modelled flux and estimate flux, and because constraining the regression through zero leads to a flux estimate that minimizes the root mean squared error of the estimated and modelled concentrations at the monitoring sites.

The ISCST3 model also requires a specially formatted meteorological data file. Meteorological data were collected onsite (see Section 7.3.2). These data were processed using the Air Pollution Model (TAPM) obtained from CSIRO Division of Atmospheric Research. TAPM is a sophisticated numerical prognostic model that is able to predict three-dimensional meteorology (Hurley, 2008). TAPM includes databases of terrain, vegetation and soil type, leaf area index, sea surface temperature and synoptic scale meteorological analysis. TAPM was used to construct an ISCST3 meteorological input file. The ISCST3 model estimates are only valid at sites off the treated field. Therefore, the on-field measurement made in this study (i.e. from station 1 see Figure 7.4) could not be used in the flux estimation.

7.3.5 Estimated buffer zones with PERFUM2

A buffer zone provides a distance between a fumigation application site (i.e., edge of the treated field) and bystanders. It is an area that allows airborne residues to disperse and reduces the health risks of bystanders being exposed to gaseous fumigants. The estimated fluxes from the indirect method (Section 7.3.4) were used in the PERFUM2 model (Reiss and Griffin, 2008) to estimate potential buffer zones for methyl iodide. PERFUM2 is routinely used in the United States to inform the decision about buffer zones.

PERFUM2 requires meteorological data, preferably over 5 years, from a representative site. Data were obtained from Coldstream weather station (where

strawberry fruit is grown) and processed using TAPM. The Coldstream weather station is located at 37.7239S and 145.4095E in Victoria, Australia.

A flux of 5 µg/m^2/sec was used for the first 12 hours after the application and a flux of 4 µg/m^2/sec was assumed for the following 12 hours in the model. These data are consistent with the fluxes calculated using the indirect method (7.3.4). The EPA reference concentration of 870 µg/m^3 for methyl iodide averaged over 24 hours was used in the analysis.

7.4 Results

7.4.1 Application conditions

In total, 210 kg of fumigant product (methyl iodide: chloropicrin 50:50) was applied in the trial, which is equivalent to an application rate of 497 kg/ha and is 99.4% of the target rate of 500kg/ha. Maximum and minimum temperatures during the trial ranged from 21ºC – 4ºC (Fig 7.5). Wind speeds ranged from 0 – 6 m/s (Fig 7.6), with an oscillating wind direction (Fig 7.7). There were three rainfall events during the trial, with a total rainfall of 25mm (Fig 7.8).

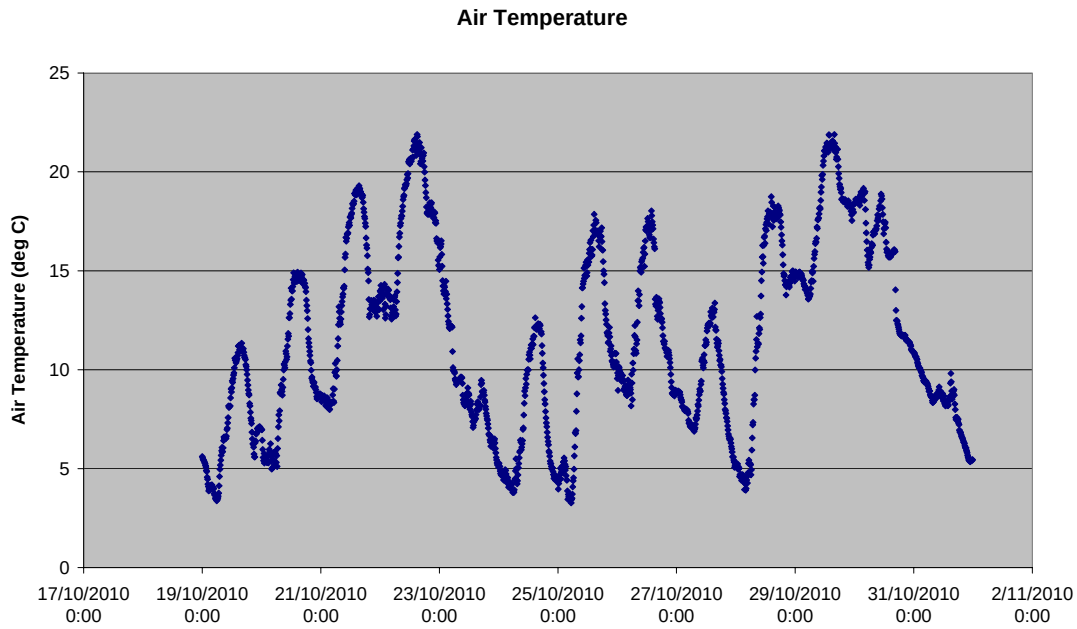


Figure 7.5. Air temperature during the trial.

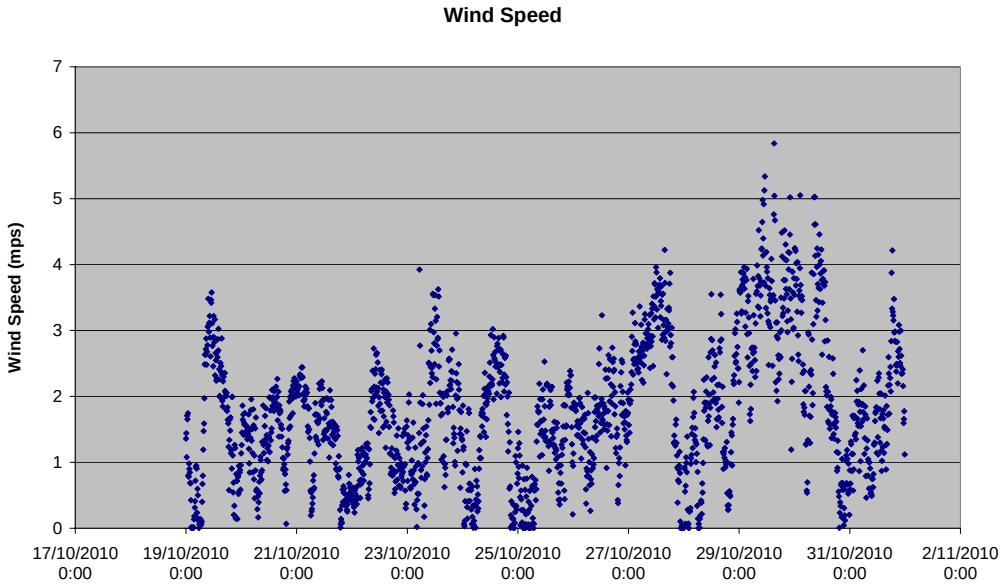


Figure 7.6. Wind speed during the trial.

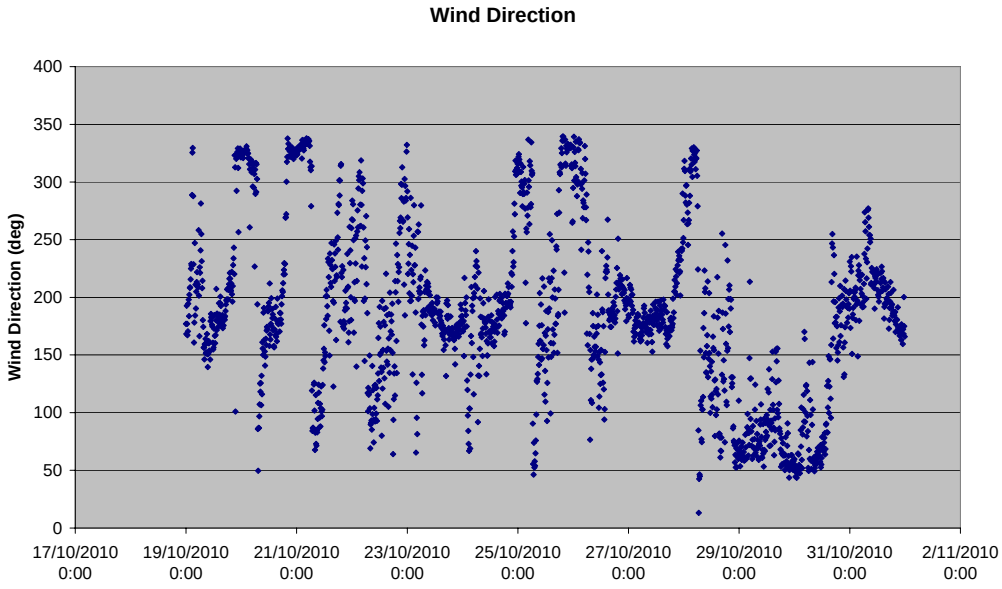


Figure 7.7. Wind direction during the trial.

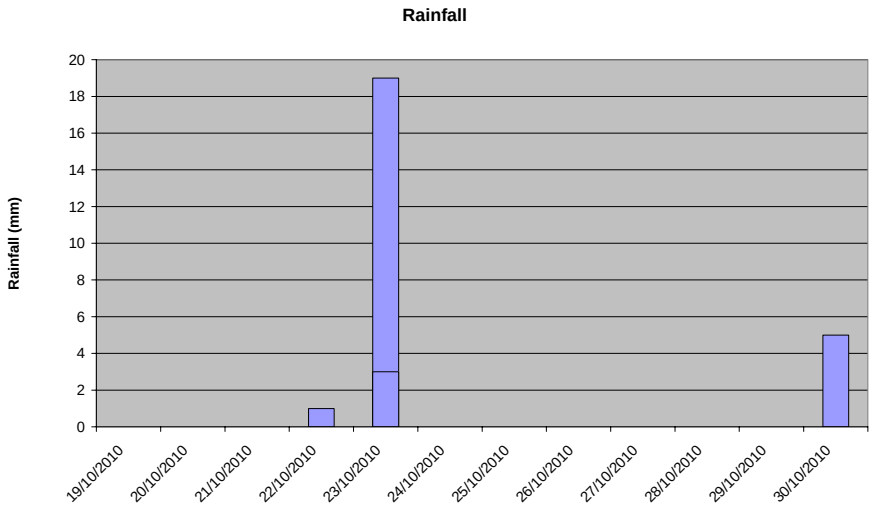


Figure 7.8. Rainfall during the trial.

7.4.2 Fumigant concentrations

Overall, the concentration of methyl iodide and chloropicrin detected in the air following fumigation was low. The maximum concentrations of methyl iodide detected in air samples 15 cm above the fumigated field was 0.71µg/L while the LDPE barrier film was in place, and 11.4µg/L following the removal of the barrier film (Table 7.3). The maximum concentrations of methyl iodide detected in air samples away from the fumigated field ranged from 0.20 – 7.9 × 10-4 µg/L (Table 7.3).

Table 7.3. Maximum concentration of methyl iodide (µg/L) detected at each air monitoring station during the trial.

Station No.	Height* (cm)	Distance † (m)	Direction†	Iodomethane concentration in air (µg/L)	
				Pre-removal of barrier film	Post-removal of barrier film
1	15	0	-	0.71 (5**)	11.4 (14)
2	15	10	S	0.18 (2)	0.20 (15)
3	180	10	S	1.8×10-2 (2)	3.9×10-2 (15)
4	180	10	S	1.6×10-2 (8)	1.8×10-2 (20)
5	180	10	W	3.0×10-2 (4)	1.9×10-2 (20)
6	180	10	W	1.9×10-2 (4)	1.9×10-2 (20)
7	180	10	N	3.0×10-2 (1)	1.6×10-2 (20)
8	180	10	N	5.2×10-2 (1)	1.9×10-2 (20)
9	180	10	E	1.8×10-2 (2)	2.1×10-2 (19)
10	180	10	E	1.4×10-2 (2)	2.3×10-2 (19)
11	15	45	SE	8.1×10-2 (7)	6.1×10-2 (19)
12	180	45	SE	1.5×10-2 (9)	2.4×10-2 (19)
13	180	45	SW	3.3×10-3 (4)	2.5×10-2 (20)
14	180	200	S	4.7×10-3 (2)	2.2×10-2 (20)
15	180	600	S	7.9×10-4 (4)	1.9×10-2 (20)

* above ground, † from the fumigated field

** sample number at which the maximum concentration of methyl iodide occurred (see Table 7.2).

The maximum concentrations of chloropicrin detected in air samples 15 cm above the fumigated field was 1.07µg/L while the LDPE barrier film was in place, and 75.94µg/L following the removal of the barrier film (Table 7.4). Chloropicrin was rarely detected in air samples away from the fumigated field. The maximum concentration of chloropicrin detected in air samples away from the fumigated field was 0.19µg/L (Table 7.4).

Table 7.4. Maximum concentration of chloropicrin (µg/L) detected at each air monitoring station during the trial.

Station No.	Height* (cm)	Distance † (m)	Direction†	Chloropicrin concentration in air (µg/L)	
				Pre-removal of barrier film	Post-removal of barrier film
1	15	0	-	1.07 (5**)	75.94 (14)
2	15	10	S	2.4×10-2 (9)	0.19 (15)
3	180	10	S	nd	1.8×10-2 (15)
4	180	10	S	nd	nd
5	180	10	W	nd	4.2×10-3 (14)
6	180	10	W	nd	3.0×10-3 (14)
7	180	10	N	nd	nd
8	180	10	N	nd	nd
9	180	10	E	nd	nd
10	180	10	E	nd	nd
11	15	45	SE	nd	nd
12	180	45	SE	nd	nd
13	180	45	SW	nd	nd
14	180	200	S	nd	nd
15	180	600	S	nd	nd

* above ground, † from the fumigated field

** sample number at which the maximum concentration of iodomethane occurred (see Table 7.2).

nd = not detected during the trial

7.4.3 Indirect fumigant fluxes

As very little chloropicrin was detected at any of the offsite monitors, no flux estimates could be made as there was insignificant offsite movement. Table 7.5 summarises the flux estimates from ISCST3 for each period after the application for methyl iodide, and the r^2 of the regression. The fluxes immediately after the application were 5.0 and 5.2 µg/m^2/sec for the first two periods. For the following day, the fluxes were 3.9 and 3.8 µg/m^2/sec before declining to lower levels until the tarp was punched. The peak flux was 7.5 µg/m^2/sec on October 25 immediately after the tarp was punched and removed. The fluxes declined thereafter, with the flux being only 0.043 µg/m^2/sec for the following period. On the last two days of the measurement period, the fluxes were about 0.25 µg/m^2/sec.

Table 7.5. Flux estimates of methyl iodide from treated soil using indirect method.

Sampling Period	Date	Start Time	Estimated Flux ($\mu g/m^2/sec$)	r2 of Regression
1	20-Oct-2010	16:00	5.0*	0.94
2	20-Oct-2010	22:00	5.2*	0.64
3	21-Oct-2010	04:00	3.9*	0.61
4	21-Oct-2010	10:00	3.8*	0.44
5	21-Oct-2010	16:00	0.80	0.087
6	21-Oct-2010	22:00	0.34*	0.74
7	22-Oct-2010	4:00	1.6	0.013
8	22-Oct-2010	10:00	1.9	0.12
9	22-Oct-2010	22:00	1.1	0.020
10	23-Oct-2010	10:00	1.6*	0.49
11	23-Oct-2010	22:00	0.51	0.15
12	24-Oct-2010	10:00	0.99*	0.37
13	24-Oct-2010	22:00	2.4*	0.72
14	25-Oct-2010	10:00	1.0	0.052
15	25-Oct-2010	22:00	7.5*	0.78
16	26-Oct-2010	10:00	0.043	0.055
17	26-Oct-2010	22:00	0.75	0.13
18	27-Oct-2010	10:00	1.6	0.078
19	27-Oct-2010	22:00	3.5	0.31
20	28-Oct-2010	10:00	1.6	0.042
21	28-Oct-2010	22:00	0.63*	0.66
22	29-Oct-2010	10:00	0.27*	0.31
23	29-Oct-2010	22:00	0.26*	0.51
24	30-Oct-2010	10:00	0.25*	0.47

*Statistically significant

Most of the regressions of the measured vs. nominal modelled concentrations were statistically significant, particularly immediately following the application when methyl iodide concentrations in air were high.

The mass of methyl iodide emitted after application can be estimated by assuming that the estimated flux for each period persists till the start of the next period and multiplying by the length of the period and the field size (0.39 ha). Table 7.6 summarises the mass emissions estimate.

Table 7.6. Estimated mass loss of methyl iodide to the atmosphere for each sampling period and the total mass lost over the experiment.

Period	Start Time	Estimated Flux ($\mu g/m^2/sec$)	Duration (hours)	Kg Emitted
1	16:00	5	6	0.42
2	22:00	5.2	6	0.44
3	4:00	3.9	6	0.33
4	10:00	3.8	6	0.32
5	16:00	0.8	6	0.07
6	22:00	0.34	6	0.03
7	4:00	1.6	6	0.13
8	10:00	1.9	6	0.16
9	22:00	1.1	12	0.19
10	10:00	1.6	12	0.27
11	22:00	0.51	12	0.09
12	10:00	0.99	12	0.17
13	22:00	2.4	12	0.40
14	10:00	1	12	0.17
15	22:00	7.5	12	1.26
16	10:00	0.043	12	0.01
17	22:00	0.75	12	0.13
18	10:00	1.6	12	0.27
19	22:00	3.5	12	0.59
20	10:00	1.6	12	0.27
21	22:00	0.63	12	0.11
22	10:00	0.27	12	0.05
23	22:00	0.26	12	0.04
24	10:00	0.25	12	0.04
			Total	6.0

7.4.4 Buffer zone determination

The PERFUM2 model estimated that no buffer zones were necessary for 5 and 10 hectare fields treated with methyl iodide: chloropicrin.

7.5 Discussion

The total estimated emissions of methyl iodide for 10 days after the application in this trial was 6.0 kg. The methyl iodide application rate was 250 kg/ha, which given the field size, represents a total of 97.6 kg applied. Thus, the 6.0 kg estimate of emissions represents about 6.1% of the applied mass. Over the first day, the estimated emissions are 1.5 kg, which represents about 1.5% of the applied mass.

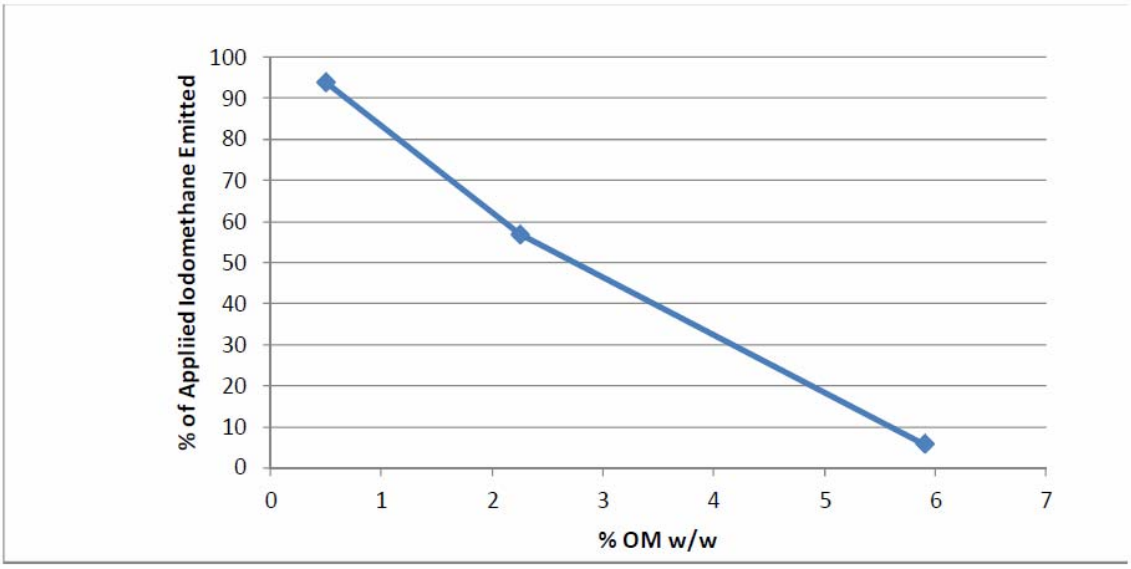
Flux estimates were not made for chloropicrin given that it was not detected in the offsite samples. The implications of these results are that emissions of methyl iodide and chloropicrin in the Toolangi environment are very low, and application of these fumigants using standard commercial methods poses minimal risks for human bystanders and the environment. Under the conditions in the current trial, no buffer zones for methyl iodide: chloropicrin would be required based on US EPA standards.

7.5.1 Comparison of methyl iodide fluxes with applications in the U.S.

Reiss and Griffin (2007) summarised the flux estimates for methyl iodide: chloropicrin under standard polyethylene tarps in California. For a broadcast application, two studies were conducted in the United States, including one in Manteca, California and one in Watsonville, California. The mass loss estimate using the indirect method for the first 24-hours was 35% at Watsonville and 51% at Manteca. For the entire monitoring after the application, the mass loss estimate was 57% at Watsonville and 93% for Manteca. These mass loss estimates were of a similar magnitude for six other applications using raised bed and drip irrigation application methods.

The mass loss estimates for the Toolangi application was 1.5% for the first 24 hours and 6.1% for the monitoring period. Thus, the Toolangi mass loss estimates are substantially lower than the estimates in the United States.

One likely explanation for the difference is the soil type. The soil at the Manteca site was sandy and the soil at the Watsonville site was a sandy loam. At Toolangi, the soil was clay. The smaller interstitial space of the clay would reasonably result in slower diffusion of gas up the soil profile resulting in lower emissions. Furthermore, the organic matter content in the Toolangi soil was high compared with the US sites (Figure 7.9). Organic matter can adsorb methyl iodide and other fumigants and reduce emissions to the atmosphere. When the percentage of emitted methyl iodide from the trials was plotted against soil organic matter there was a direct linear relationship (Figure 7.9). This adds considerable weight to the importance of soil organic matter on emissions of methyl iodide.



	Manteca	Watsonville	Toolangi
Organic matter	0.50%	2%	6%
Average soil temperature (range)	26.5 (17 – 40) C	(21-25) C	(19.5-22) C
Iodomethane mass loss*	94.00%	57%	6%

Figure 7.9. Relationship between emission of methyl iodide to the atmosphere and soil organic matter in three bystander safety trials conduced in the US and Australia.

7.5.2 Comparison of methyl iodide concentrations with the U.S. EPA toxicity level

The concentrations of methyl iodide that were measured at the offsite monitors (n=324) in the Toolangi trial were all below the EPA reference concentration (toxicity level including safety factors) of 870 µg/m3. Figure 7.10 shows a plot of the distribution of concentrations at the stations 10 meters from the field (stations 2-10) along with the EPA toxicity level. The maximum concentration was 199 µg/m3 and the 95th percentile concentration was 109 µg/m3 among the 207 measurements at 10 meters from the field. The maximum concentration was more than 4-fold below the EPA toxicity level, and the 95th percentile concentration was more than 8-fold below the EPA toxicity level. This shows that, even at a very small distance from the field (10 metres), the concentrations of methyl iodide did not approach the EPA toxicity level.

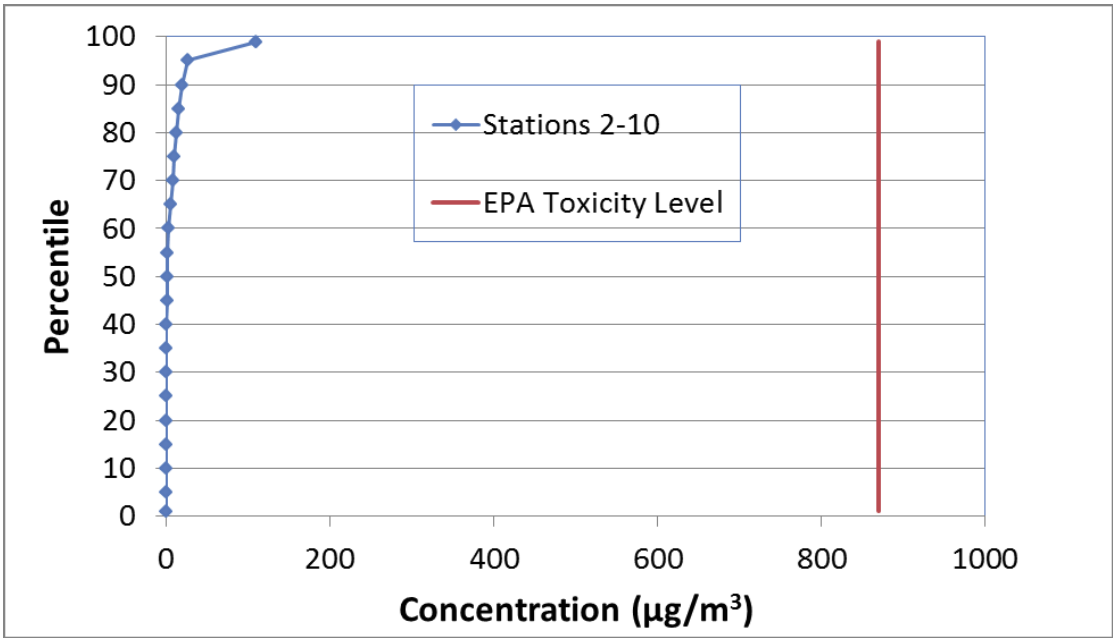


Figure 7.10. Distribution of measured concentrations of methyl iodide at Toolangi at station 10 m from the treated plot, compared with the EPA reference concentration.

8. New MB Alternatives (Activity 6)

8.1 Introduction

This chapter summarises trials investigating the efficacy of recaptured methyl bromide from quarantine and pre-shipment (QPS) applications for soil disinfestation in the strawberry runner industry. Currently, the strawberry runner industry relies on soil disinfestation with methyl bromide to maintain biosecurity standards and market access for strawberry transplants sent around Australia. However, methyl bromide is an ozone depleter and is being phased-out under the terms of the *Montreal Protocol*. QPS uses of methyl bromide are not currently regulated under the *Montreal Protocol*. Conceptually, therefore, the strawberry runner industry could legitimately use recaptured methyl bromide from quarantine applications for soil disinfestation and as an alternative to importing 30 tonnes of methyl bromide p.a. specifically for soil uses. Three completed trials in the runner industry showed that recaptured methyl bromide from quarantine applications, co-applied with chloropicrin, provided equivalent pathogen and weed control and runner yields to traditional formulations of methyl bromide. Additionally, the use of recaptured methyl bromide may offer other benefits, including reduced emissions of methyl bromide to the atmosphere, sequestration of carbon into agricultural soils and accelerated recolonisation of soils by microflora.

8.2 Materials and method

8.2.1 Trial 1 (2008/09)

The trial was conducted at Toolangi, Victoria (37º32’07.18’’S, 145º27’27.92’’E, 430 m above sea level) on a krasnozem soil with a silty-clay texture in a field adjacent (within 20 m) to a commercial runner production area. The site had no history of fumigation or strawberry production, and had a gentle slope (< 10º) running from west to east. The site was prepared for fumigation by rotary hoeing to a depth of 20 cm.

QPS MB was applied (26 November, 2008) by spreading carbon containing recaptured methyl bromide over the soil, rotary hoeing it to a depth 20 cm, and laying low-density polyethylene (35 μm thick) over the soil as a surface seal. All other fumigant treatments (Table 8.1) were applied by shank-injection to a soil depth of 20 cm under low-density polyethylene. Treatments were applied in 2.7 m wide strips with a commercial fumigation rig (by R&R Fumigation Services) through shanks spaced 25 cm apart. Seven days after fumigation (3 December, 2008), LDPE film was cut and removed, and soil allowed to air for a further three weeks before planting. Four weeks after fumigation (23 December, 2008), plots were planted with a single row of strawberry mother plants (bare-rooted), var. Gaviota, spaced 50 cm apart. Planting was made by hand using dibble trowel. All other agronomic procedures (e.g. irrigation, fertilisation) followed standard industry practices for strawberry runner production.

Table 8.1: Fumigant treatments applied in a QPS MB field trial in the strawberry runner industry in Toolangi, Vic in 2008/2009.

Fumigant Treatment	Treatment Code	Application Rate	Application Method	Rationale
Recaptured methyl bromide from QPS treatments	QPS MB	5000 kg/ha of activated carbon (7% methyl bromide) = 350 kg ai/ha	Incorporation to 20 cm with rotary hoe followed by soil sealing with LDPE film	Experimental treatment and rate
Methyl bromide: chloropicrin (50:50)	MB500	500 kg/ha	Shank injection under LDPE film	Positive control: standard methyl bromide treatment
Methyl bromide: chloropicrin (50:50)	MB250	250 kg/ha	Shank injection under LDPE film	Lower rate of methyl bromide proposed as a possible emission control strategy
Methyl iodide : chloropicrin (50:50)	MI350	350 kg/ha	Shank injection under LDPE film	Leading alternative to methyl bromide applied at proposed label rate.
Untreated	Untreated	Nil	LDPE film only	Negative control

Methyl bromide concentration in soil

The concentration of methyl bromide in soil air was determined in QPS MB, MB250, MB500 treatments and the untreated control over time. In this procedure, a soil probe (Nicholls et al., 1999) was inserted into the middle of the plot to a depth of 10 cm. Soil air (40 ml) was drawn through the probe onto a carbon sampling tube (Orbico 32) using a syringe. Methyl bromide was eluted from the carbon tube with carbon disulphide. The concentration of methyl bromide in the sample was determined using GC/MS (HP 5970B MSD) using the method described by Nicholls et al. (1999). Measurements were taken 6, 24, 120, and 288 hours after fumigation. Exposure to methyl bromide (i.e. concentration × time) was calculated over a period 2-weeks as the area under the fumigant concentration/time curve (see Figure 8.1).

Pathogen viability

Immediately following fumigant application, four muslin bags containing 1 g of inoculum of the strawberry pathogens *Phytophthora cactorum* and *Rhizoctonia fragariae* were buried per plot at depths of 10 cm. Inoculum of *P. cactorum* was grown in a V8/vermiculite medium, while *R. fragariae* was grown on double autoclaved (121ºC, 20 min) millet seed. Five days after fumigation, the muslin bags were retrieved and 10 pieces of inoculum per bag plated onto *Phytophthora* selective media (corn meal agar amended with 250 µL/L pimaricin, 0.25 g/L ampicillin, 0.01

g/L rifampicin and 0.1 g/L PCNB) or potato dextrose agar amended with 0.05 g/L achromycin (PDA+A) to determine their viability.

Pythium concentration
Four weeks after fumigation (at planting), a motorised auger (80-mm diameter) was used to take 10 random (W sampling pattern) soil samples per plot at depths of 10 cm, 30 cm and 80 cm. Soil samples from each depth were pooled and a 500 g sub-sample taken. Concentrations of *Pythium* spp. (clade F, which contains species pathogenic to strawberry) were determined in the soil sub-samples using q-PCR, conducted by a commercial laboratory (SARDI).

Runner establishment
Early vegetative growth of strawberry mother plants was determined one month after planting (21 January, 2009). Five random mother plants per plot were measured for length of the primary stolon (using a ruler), total stolon number and leaf number.

Weed emergence
Three months after fumigation (25 February, 2009) the number and identity of weeds emerging in five random 0.016 m² quadrats per plot were determined.

Runner yield
Final yields were determined seven months after planting (22 July, 2009) by digging and then counting all runners contained within two random 0.5 m lengths of row per plot. Additionally, crown diameters (measured with a calliper) of 10 randomly selected runners per plot were taken.

Disease incidence
Following harvest, the roots of 10 random runners per plot (the same runners taken for crown diameter samples) were washed free of soil and scored for black root severity using the scale in Table 8.2. Additionally, the incidence of crown rot was determined in the same runners by cutting crowns and examining them for characteristic symptoms caused by *Phytophthora cactorum*.

Table 8.2. Assessment chart for black root severity (Mattner et al., 2002).

Black Root Score	Description
0	Healthy roots
1	< 5% severity of brown lesions on roots
2	< 5% severity of black lesions on roots
3	> 5% severity of brown lesions on roots
4	> 5 % severity of black lesions on roots
5	Roots 100% black

The trial was conducted as a randomised complete block design with three blocks. Treatments are listed in Table 8.1. Data were analysed using ANOVA as performed on Genstat v. 12 (Lawes Agricultural Trust, IACR Rothamsted). Homogeneity of variance was determined by examining plots of fitted values and residuals, while histograms of residuals assessed normality of distribution. Where variance was heterogeneous, appropriate data transformations were made to restore homogeneity.

Fischer's least significance difference test (LSD, p = 0.05) was used to identify significant differences between treatment means.

8.2.2 Trial 2 (2009/10)

The trial was established adjacent (37º32'05.91''S, 145º27'28.58''E) to the site described in trial 1, and consisted of the same soil type and cropping history. The trial site was prepared for fumigation by rotary hoeing to a depth of 20 cm.

QPS MB was applied (2 December, 2009) by spreading carbon containing recaptured methyl bromide over the soil, rotary hoeing it to a depth 20 cm, and laying low-density polyethylene (35 μm thick) over the soil as a surface seal. Additionally, chloropicrin (CP, 250 kg/ha) was shank-injected into half of the plots amended with QPS MB to form a QPS MB/CP treatment. All other fumigant treatments (Table 8.3) were applied by shank-injection to a soil depth of 20 cm under low-density polyethylene. Treatments were applied in 2.7 m wide strips with a commercial fumigation rig (by R&R Fumigation Services) through shanks spaced 25 cm apart.

Seven days after fumigation (9 December, 2009), LDPE film was cut and removed, and soil allowed to air for a further three weeks before planting. Four weeks after fumigation (30 December, 2009), plots were planted with a single row of strawberry mother plants (bare-rooted), var. Gaviota, spaced 50 cm apart. Planting was made by hand using a dibble trowel. All other agronomic procedures (e.g. irrigation, fertilisation) followed standard industry practices for strawberry runner production.

Fumigant concentration in soil air

The concentration of methyl bromide and chloropicrin in soil air were determined in QPS MB, QPS MB/CP, CP and MB500 treatments, and the untreated control over time. In this procedure, a soil probe was inserted into the middle of the plot to depths of 10 cm and 20 cm. Soil air (40 ml) was drawn through the probe using a syringe onto a carbon sampling tube. Fumigants were later extracted and concentrations determined using GC/MS as previously described. Measurements were taken at 4, 48, and 168 hours after fumigation. Exposure to methyl bromide and chloropicrin (i.e. concentration × time) was calculated over a period of seven days as the area under the fumigant concentration/time curves.

Methyl bromide emissions

Emissions of methyl bromide through the LDPE seal were measured at 2, 5 and 7 days after fumigation in QPS MB/CP and MB500 treatments only. In this procedure, a lidded chamber (20 cm diameter, 2 L volume) was attached to the LDPE film using silicon and duct tape. At each sampling time, the lid was secured onto the chamber for a period of 1-hr. After this period 40 ml of air within the chamber was sampled through a septum in the lid and drawn onto a carbon sampling tube. Methyl bromide was later extracted from the tube and the concentration determined using GC/MS as previously described. The emission rate of methyl bromide through the LDPE film was calculated based on the concentration within the chamber, the area and volume of the chamber, and sampling time. This method follows a similar procedure to that used for determining greenhouse gas emissions from soil (Weier, 1999).

Table 8.3. Fumigant treatments applied in a QPS MB field trial in the strawberry runner industry in Toolangi, Vic in 2009/2010.

Fumigant Treatment	**Treatment Code**	**Application Rate**	**Application Method**	**Rationale**
Recaptured methyl bromide from QPS applications	QPS MB	3572 kg/ha of activated carbon (7% methyl bromide) = 250 kg ai/ha	Incorporation to 20 cm with a rotary hoe followed by soil sealing with LDPE film	Applied at equivalent rates of MB to shank-injected MB500
Recaptured methyl bromide from QPS applications, co-applied with chloropicrin	QPS MB/CP	3572 kg/ha of activated carbon (7% methyl bromide) = 250 kg ai/ha + 250 kg/ha chloropicrin	Incorporation of QPS MB to 20 cm with a rotary hoe followed by shank injection of CP under LDPE film	Applied at equivalent rates of MB and CP to shank-injected MB500
Chloropicrin	CP	250 kg/ha	Shank injection under LDPE film	Applied at equivalent rates of CP to shank-injected MB500
Methyl bromide: chloropicrin (50:50)	MB500	500 kg/ha (250 kg/ha MB and 250 kg/ha CP)	Shank injection under LDPE film	Positive control: standard methyl bromide treatment
Methyl bromide: chloropicrin (50:50)	MB400	400 kg/ha	Shank injection under LDPE film	Lower rate of methyl bromide proposed as a possible emission control strategy
Methyl iodide : chloropicrin (50:50)	MI350	350 kg/ha	Shank injection under LDPE film	Leading non-registered alternative to methyl bromide applied at proposed label rate
1,3 dichloropropene : chloropicrin (65:35)	TC35	500 kg/ha	Shank injection under LDPE film	Leading registered alternative to methyl bromide applied at label rate
Untreated	Untreated	Nil	LDPE film only	Negative control

Pathogen viability
Immediately following fumigant application, four muslin bags containing 20 sclerotes or microsclerotes of the strawberry pathogens *Sclerotium rolfsii* and *Verticillium dahliae* were buried per plot at depths of 10 cm. Five days after fumigation, the muslin bags were retrieved and 10 sclerotes surface sterilised and plated onto potato dextrose agar amended with 0.05 g/L achromycin (PDA+A) to determine their viability.

Microbial activity in soil
Soil microbial activity was measured at 1, 2, 4, 8 and 16 weeks after fumigation using the method described by Schnurer and Rosswall (1982) based on the hydrolysis of fluorescein diacetate. This assay measures the presence of universal enzymes (e.g. lipases, proteases, etc) of biological origin and function in soil, and is related to total microbial activity.

Soil chemistry
At 1, 2, and 4 weeks after treatment, a hand auger was used to take 10 random (W sampling pattern) soil samples per plot at depths of 0-10 cm. Soil samples were pooled a sub-sample of 500 g submitted to a commercial laboratory (SWEP, Victoria) for standard nutrient analyses.

Pythium concentration
Four weeks after fumigation (at planting), soils from each plot were sampled as described above and submitted to a commercial laboratory (SARDI) for analysis of concentrations of *Pythium* spp. (clade F, which contains species pathogenic to strawberry) using q-PCR.

Weed emergence
Three months after fumigation (24 February, 2010) the number and identity of weeds emerging in five random 0.016 m² quadrats per plot were determined.

Runner yield
Final yields were determined seven months after planting (7 July, 2010) by digging and then counting all runners contained within two random 0.5 m lengths of row per plot. Additionally, crown diameters (measured with a calliper) of 10 randomly selected runners per plot were taken.

Disease incidence
Following harvest, the roots of 10 random runners per plot (the same runners taken for crown diameter samples) were washed free of soil and scored for black root severity using the scale in Table 2. Additionally, the incidence of crown rot was determined by cutting crowns and examining them for characteristic symptoms caused by *Phytophthora cactorum*.

The trial was conducted as a randomised complete block design with four blocks. Treatments are listed in Table 8.3. Data were analysed using ANOVA as performed on Genstat v. 12 (Lawes Agricultural Trust, IACR Rothamsted). Homogeneity of variance was determined by examining plots of fitted values and residuals, while histograms of residuals assessed normality of distribution. Where variance was heterogeneous, appropriate data transformations were made to restore homogeneity.

Fischer's least significance difference test (LSD, p = 0.05) was used to identify significant differences between treatment means.

8.2.3 Trial 3 (2011/12)

The trial was conducted in sand beds at DPI, Knoxfield, Victoria. The site was prepared for fumigation by rotary hoeing to a depth of 20 cm and wetting the beds overnight to attain optimum moisture.

Soils in the beds were inoculated with the fungus *Rhizoctonia fragariae* AG I (note there is currently no valid name for this organism, Anderson and Staplers, 1994), which can be a weak pathogen of strawberry and contribute to causing the disease complex black root rot. In this procedure plugs of the actively growing fungus (on PDA) were added to double autoclaved (121ºC, 20 min), moisten millet seed. Colonised millet seed was incorporated to a depth of 10 cm into the planting rows in the beds at a rate of 200 mL of inoculum / m of row. The fungus was allowed to colonise the soil for a period of 1-week before treatment.

QPS MB was applied by spreading carbon containing recaptured methyl bromide over the soil, rotary hoeing it to a depth 20 cm, and laying low-density polyethylene (LDPE) or virtually impermeable film (VIF, Bromostop®) over the soil as a surface seal. Dazomet was spread over the sand and rotary hoed, watered in, and then sealed with LDPE. The untreated plots were rotary hoed, watered in, and also sealed with LDPE.

Seven days after fumigation films were cut and removed, and sand allowed to air for a further three weeks before planting. Plots were planted with a single row of strawberry mother plants (bare-rooted), var. Gaviota, spaced at 50 cm apart. Planting was made by hand using dibble trowel. All other agronomic procedures (e.g. irrigation, fertilisation) followed standard industry practices for strawberry runner production.

Runner yield

Final yields were determined seven months after planting by digging and then counting all runners contained within two random 0.5 m lengths of row per plot.

Disease incidence

Following harvest, the roots of 10 random runners per plot were washed free of soil and scored for black root severity using the scale in Table 8.2.

The trial was conducted as a randomised complete block design with four blocks. Treatments are listed in Table 8.4. Data were analysed using ANOVA as performed on Genstat v. 12 (Lawes Agricultural Trust, IACR Rothamsted). Homogeneity of variance was determined by examining plots of fitted values and residuals, while histograms of residuals assessed normality of distribution. Where variance was heterogeneous, appropriate data transformations were made to restore homogeneity. Fischer's least significance difference test (LSD, p = 0.05) was used to identify significant differences between treatment means.

Table 8.4 Fumigant treatments applied in a replicated sandbed trial at Knoxfield, Vic in 2010-2012.

Fumigant Treatment	Treatment Code	Application Rate	Application Method	Rationale
Recaptured methyl bromide from QPS treatments	QPS MB100	1430 kg/ha of activated carbon (7% methyl bromide) = 1000 kg ai/ha	Incorporation to 20 cm with rotary hoe followed by soil sealing with LDPE film	Experimental treatment and rate
Recaptured methyl bromide from QPS treatments	QPS MB75	1072 kg/ha of activated carbon (7% methyl bromide) = 750 kg ai/ha	Incorporation to 20 cm with rotary hoe followed by soil sealing with LDPE film	Experimental treatment and rate
Recaptured methyl bromide from QPS treatments	QPS MB50	715 kg/ha of activated carbon (7% methyl bromide) = 500 kg ai/ha	Incorporation to 20 cm with rotary hoe followed by soil sealing with LDPE film	Experimental treatment and rate
Recaptured methyl bromide from QPS treatments	QPS MB25	360 kg/ha of activated carbon (7% methyl bromide) = 250 kg ai/ha	Incorporation to 20 cm with rotary hoe followed by soil sealing with LDPE film	Experimental treatment and rate
Recaptured methyl bromide from QPS treatments	VIF QPS MB100	1430 kg/ha of activated carbon (7% methyl bromide) = 1000 kg ai/ha	Incorporation to 20 cm with rotary hoe followed by soil sealing with VIF film	Experimental treatment and rate
Recaptured methyl bromide from QPS treatments	VIF QPS MB75	1072 kg/ha of activated carbon (7% methyl bromide) = 750 kg ai/ha	Incorporation to 20 cm with rotary hoe followed by soil sealing with VIF film	Experimental treatment and rate
Recaptured methyl bromide from QPS treatments	VIF QPS MB50	715 kg/ha of activated carbon (7% methyl bromide) = 500 kg ai/ha	Incorporation to 20 cm with rotary hoe followed by soil sealing with VIF film	Experimental treatment and rate
Recaptured methyl bromide from QPS treatments	VIF QPS MB25	360 kg/ha of activated carbon (7% methyl bromide) = 250 kg ai/ha	Incorporation to 20 cm with rotary hoe followed by soil sealing with VIF film	Experimental treatment and rate
Dazomet	DAZ	500 kg/ha	Incorporation to 20 cm with rotary hoe followed by soil sealing with LDPE film	Positive control
Untreated	Untreated	Nil	LDPE film only	Negative control

8.3 Results

8.3.1 Trial 1 (2008/09)

Methyl bromide concentration

Methyl bromide concentrations in soils treated with QPS MB were significantly lower (by up to 50%) than in plots treated with MB500 or MB250, particularly early after treatment (Figure 8.1). No detectable concentrations of methyl bromide occurred in plots treated with QPS MB at two-weeks after treatment. By comparison, concentrations of methyl bromide in shank-injected treatments (MB250 and MB500) at this time averaged 40 ppm. Concentrations of methyl bromide were not detected in untreated plots at any sampling time.

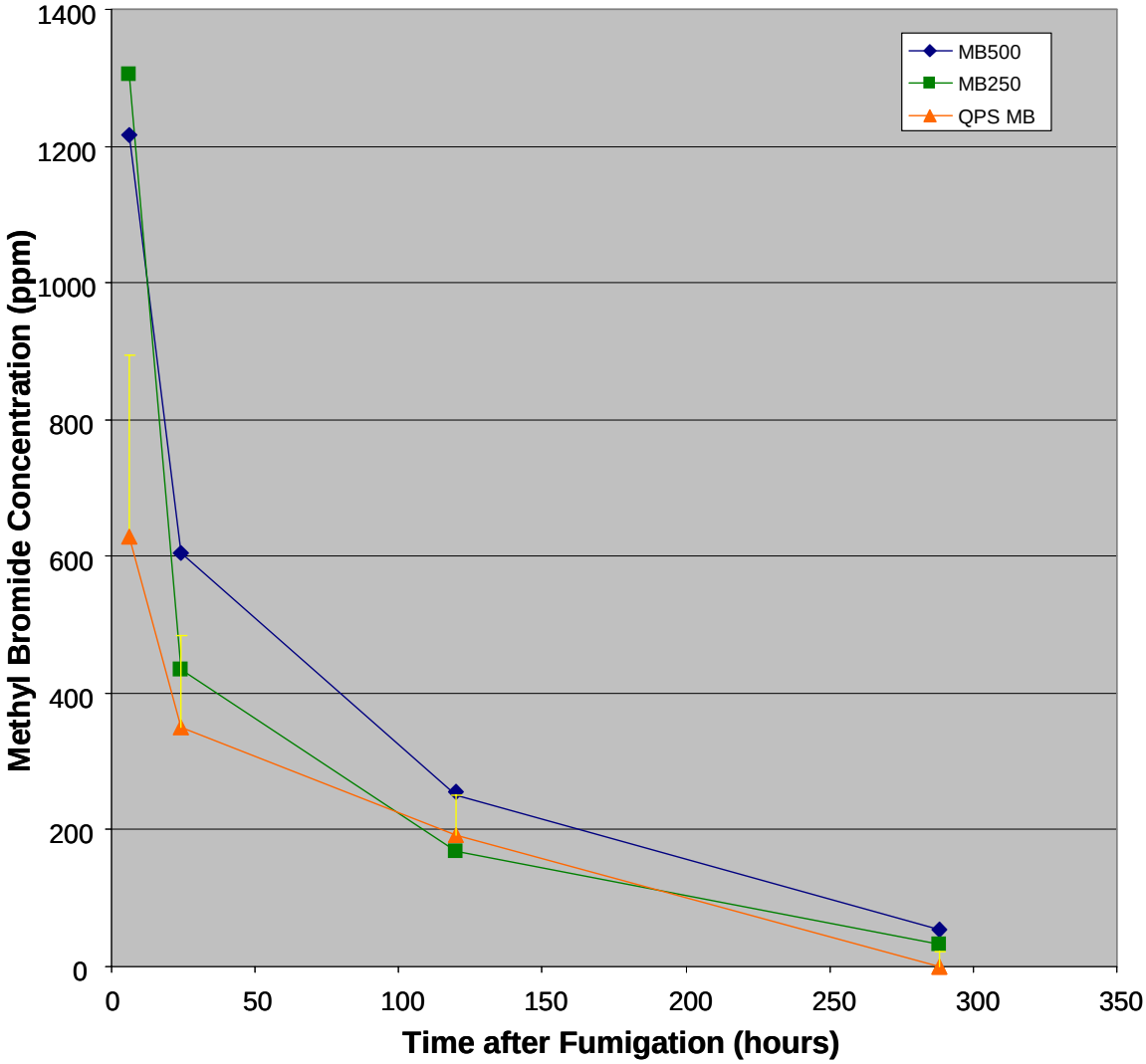


Figure 8.1. Concentration (ppm) of methyl bromide in soil air following treatment with three fumigants in a field trial at Toolangi, Vic in 2008/09. Bars are LSDs where p = 0.05.

Overall, exposure to methyl bromide in plots treated with QPS MB was significantly lower, by 20% and 40%, than in plots treated with MB250 and MB500, respectively (Table 8.5).

Table 8.5. Exposure of soil to methyl bromide over a 2 week period in a field trial at Toolangi, Vic in 2008/09.

Fumigant Treatment	Exposure to MB (ppm.hr)
Untreated	0
QPS MB	53,960
MB250	66,717
MB500	88,470
LSD (p = 0.05)	**12,147**

Pathogen viability

All fumigants, including QPS MB, reduced the viability of artificial inoculum of *Phytophthora cactorum* and *Rhizoctonia fragariae* from 72% and 86% (as recorded the untreated control), respectively, to nil.

Pythium concentration

Overall, concentrations of *Pythium* spp. in soil were significantly lower at 30 cm and 80 cm depth than at 10 cm (Table 8.6).

Table 8.6. Average Pythium (clade F) concentrations at different soil depths in a field trial at Toolangi, Vic in 2008/09. Values followed by different letters are significantly different, where p = 0.05. Data was analysed following a $\log_{10}$ (x+0.1) transformation.

Soil Depth	*Pythium* Concentration (pg DNA/g soil)
10 cm	58.6 a
30 cm	5.6 b
80 cm	13.3 b

Treatment with QPS MB showed a trend towards reducing the concentration of *Pythium* in soil compared with the untreated control, but this was not significant (Table 8.7). All other fumigants significantly reduced concentrations of *Pythium* in soil compared with the untreated control. There was no significant interaction between soil depth and fumigant treatment.

Table 8.7. Average Pythium (clade F) concentrations in soils treated with different fumigants in a field trial at Toolangi, Vic in 2008/09. Values followed by different letters are significantly different, where p = 0.05. Data was analysed following a $\log_{10}$ (x+0.1) transformation.

Fumigant	*Pythium* Concentration (pg DNA/g soil)
Untreated	87.6 a
QPS MB	31.5 ab
MI350	1.8 c
MB250	5.4 bc
MB500	2.8 c

Runner establishment

The primary stolons of mother plants grown in soil treated with QPS MB were significantly longer (by 23%) than those of plants grown in untreated control. Furthermore, plants grown in QPS MB-treated soil had equivalent primary stolon lengths to plants grown in MB250- and MI350-treated soils. However, stolon lengths of plants grown in plots treated with MB500 were significantly (by 13%) longer than those of plants in the QPS MB treatment (Table 8.8).

There was a trend towards mother plants grown in fumigated soils having more stolons and leaves than plants grown in untreated soils, but this was not statistically significant (Table 8.8).

Table 8.8. Growth parameters of strawberry mother plants establishing in soils treated with various fumigants in a field trial at Toolangi, Vic in 2008/09.

Fumigant	Length of Primary Stolon (mm)	Stolons per Plant	Leaves per Plant
Untreated	196.6	2.00	4.13
QPS MB	242.2	2.33	4.46
MI350	266.7	2.46	4.60
MB250	255.6	2.46	4.46
MB500	278.9	2.53	4.46
LSD (p = 0.05)	29.4	ns	ns

Weed emergence

Chenopodium ambrosioides (Mexican tea) (55%), *Echinochloa crus-galli* (barnyard grass) (11%), *Chenopodium album* (fat hen) (8%), and *Raphanus raphanistrum* (wild radish) (7%) were the dominant weeds to emerge on the site.

Soil treatment with QPS MB significantly reduced (by 80%) total weed emergence compared with the untreated control. This level of weed control was statistically equivalent to that in MI350 plots. However, weed emergence in soils treated with shank injected methyl bromide (MB250 and MB500) was significantly lower (by an average of 85%) than that in the QPS MB treatment (Figure 8.2).

Runner yields

There was a trend towards runners grown in soils treated with QPS MB to have higher yields than those grown in untreated soil, but this was not statistically significant. By comparison, strawberries grown in soil treated with shank-injected fumigants (MI350, MB250, and MB500) had significantly higher yields (by an average of 80%) than those grown in untreated soils. Runner yields in QPS MB and shank-injected treatments were statistically equivalent (Table 8.9). The average crown diameter of runners showed a similar response to fumigant treatments as runner yield (Table 8.9).

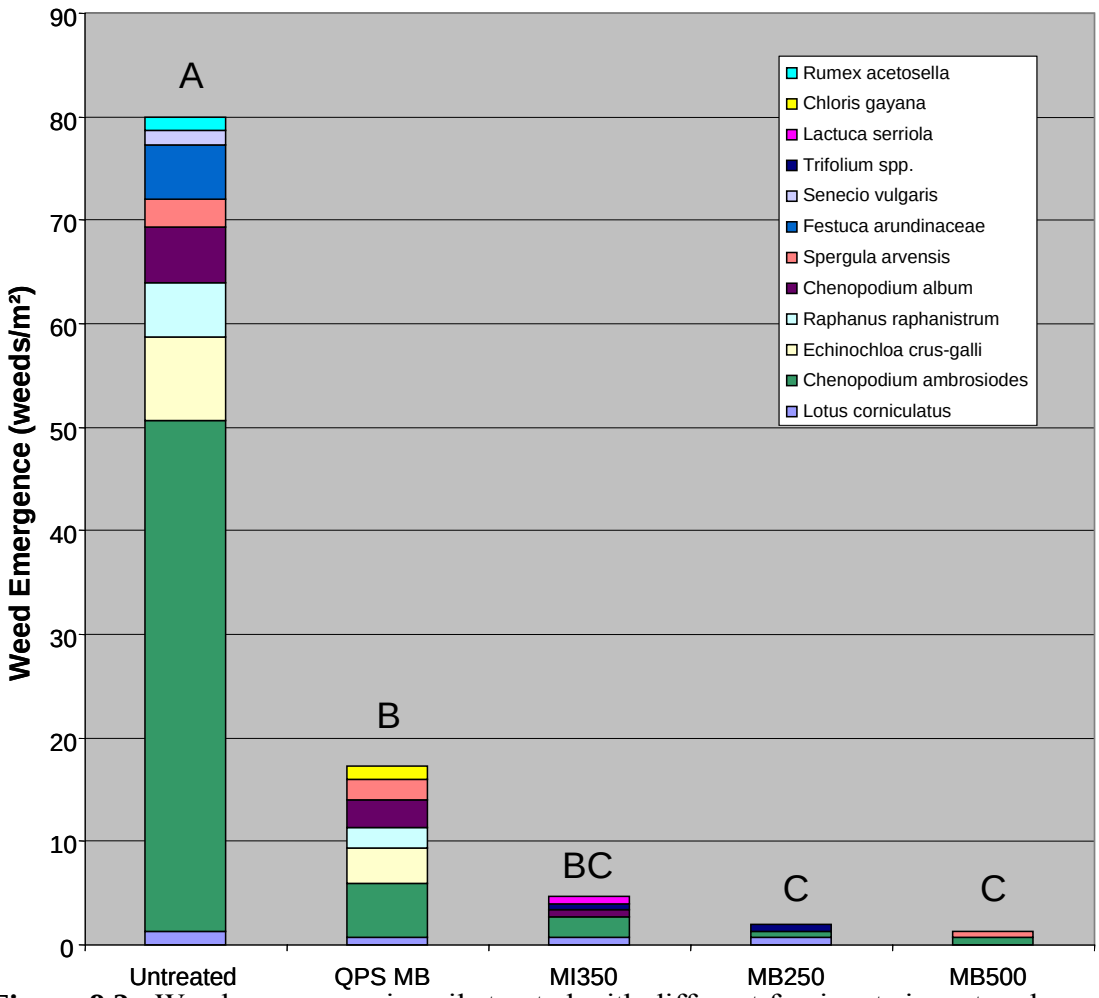


Figure 8.2. Weed emergence in soils treated with different fumigants in a strawberry field trial at Toolangi, Vic in 2008/09. Bars followed by different letters are significantly different, where p = 0.05. Analysis was made on $\log_{10}(x+1)$ transformed data.

Table 8.9. Final yields and crown diameters of strawberry runners grown in various fumigant treatments in a field trial at Toolangi, Vic in 2008/09.

Fumigant	**Runner Yield (plantlets/m)**	**Crown Diameter (mm)**
Untreated	49.0	11.5
QPS MB	74.3	12.8
MI350	90.0	13.5
MB250	90.3	13.6
MB500	87.0	13.3
LSD (p = 0.05)	37.8	1.4

Black root score and crown rot incidence

Soil treatment with all fumigants reduced the severity of black roots in harvested runners (Table 8.10). The incidence of crown rot caused by *Phytophthora cactorum* in the trial was low and could not be statistically analysed.

Table 8.10. Black root score and crown rot incidence in strawberry runners grown in soils treated with various fumigants in a field trial at Toolangi, Vic in 2008/09.

Fumigant	Black Root Score (0-5)	Incidence of Crown Rot (%)
Untreated	1.20	1.3
QPS MB	0.56	0.3
MI350	0.40	0.0
MB250	0.53	0.0
MB500	0.33	0.0
LSD (p = 0.05)	0.29	-

8.3.2 Trial 2 (2009/10)

Fumigant concentration in soil air

Methyl bromide concentrations in soils treated with QPS MB were significantly lower (by up to 50%) than in plots treated with MB500 (Figure 8.3). Concentrations of chloropicrin were significantly lower in plots treated with QPS MB/CP than in the CP or MB500 treatments at the 4 hour measurement only (Figure 8.4). Concentrations of methyl bromide or chloropicrin were not detected in untreated plots at any sampling time. There was a trend towards lower exposures to methyl bromide and chloropicrin (Table 8.11) in the QPS MB/CP treatment, but this was not statistically significant.

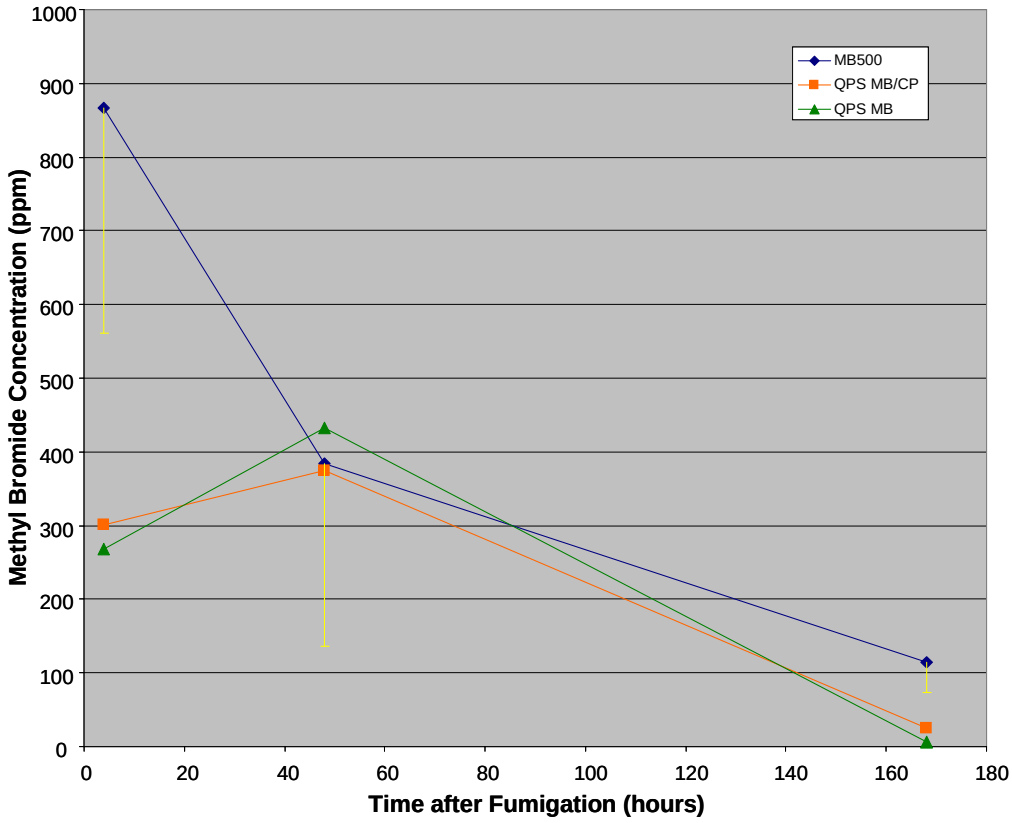


Figure 8.3. Concentration (ppm) of methyl bromide in soil air treated with three fumigants in a field trial at Toolangi, Vic in 2009/10. Bars are LSDs where p = 0.05.

Table 8.11. Exposure of soil to methyl bromide and chloropicrin over a 7-day period in a field trial at Toolangi, Vic in 2009/10.

Fumigant	Exposure to MB (ppm.hr)	Exposure to CP (ppm.hr)
Untreated	0	0
QPS MB	43,173	-
QPS MB/CP	40,241	9,828
MB500	59,900	18,590
CP	-	19,637
LSD (p = 0.05)	**ns**	**ns**

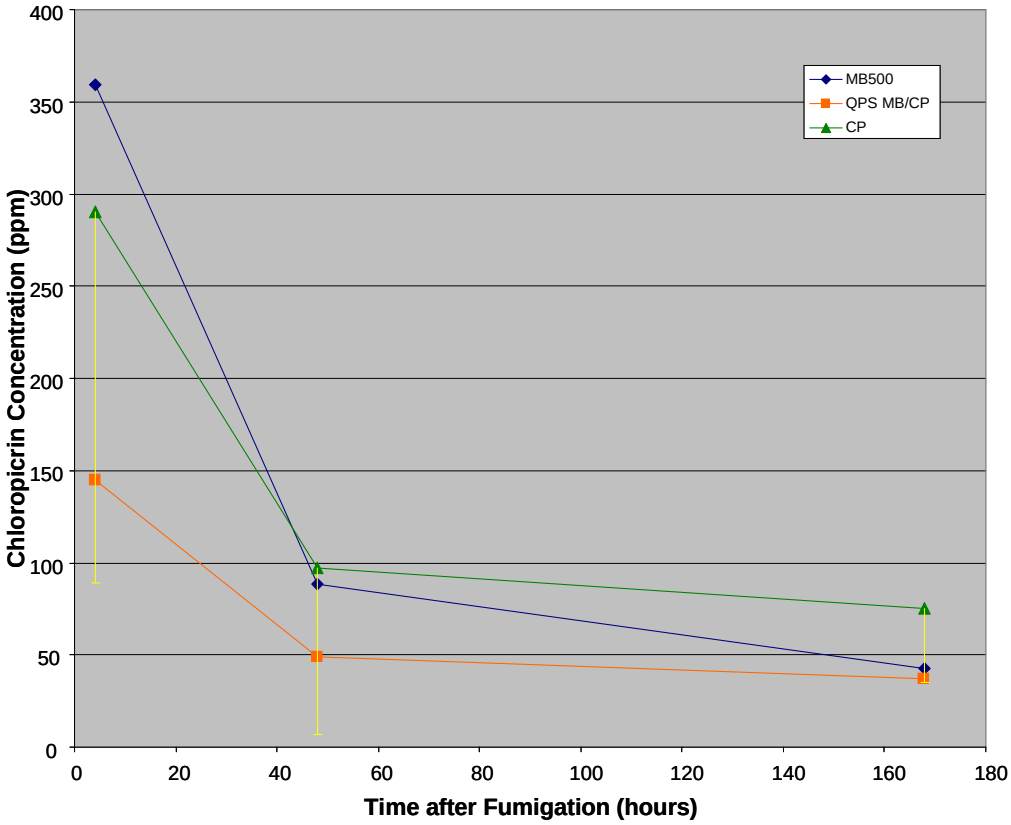


Figure 8.4. Concentration (ppm) of chloropicrin in soil air treated with three fumigants in a field trial at Toolangi, Vic in 2009/10. Bars are LSDs where p = 0.05.

Methyl bromide emissions

There was consistent trend towards lower emissions of methyl bromide through LDPE from plots treated with QPS MB/CP than through the shank-injected treatment (MB500), but this was not statistically significant (Figure 8.4).

Pathogen viability

All fumigants, including QPS MB/CP, reduced the viability of sclerotia and microsclertoia of *Sclerotium rolfsii* and *Verticillium dahliae* from 89% and 93% (in the untreated control), respectively, to zero or low levels (6.8% and 2.5% of sclerotia of *S. rolfsii* were viable in the TC35 and MB400 treatments, respectively).

Microbial activity in soil

At one week after treatment, all fumigants reduced microbial activity in soil compared with the untreated control (Table 8.12). QPS MB/CP and MB500 reduced microbial activity to equivalent levels (by 40% compared with the untreated control). Co-application with CP significantly reduced activity in soils treated with QPS MB.

At two and four weeks after treatment, all fumigants reduced microbial activity in the soil, compared with the untreated control, to equivalent levels (by an average of 40%) (Table 8.12).

By 16-weeks, microbial activity in treatments containing QPS MB had recovered to equivalent levels as in the untreated control. In contrast, microbial activity in soils treated with other fumigants was still significantly below that in the untreated control. In particular, soils treated with TC35 had low microbial activity, which probably reflects the long residual times of 1,3-dichloropropene in soil (Table 8.12).

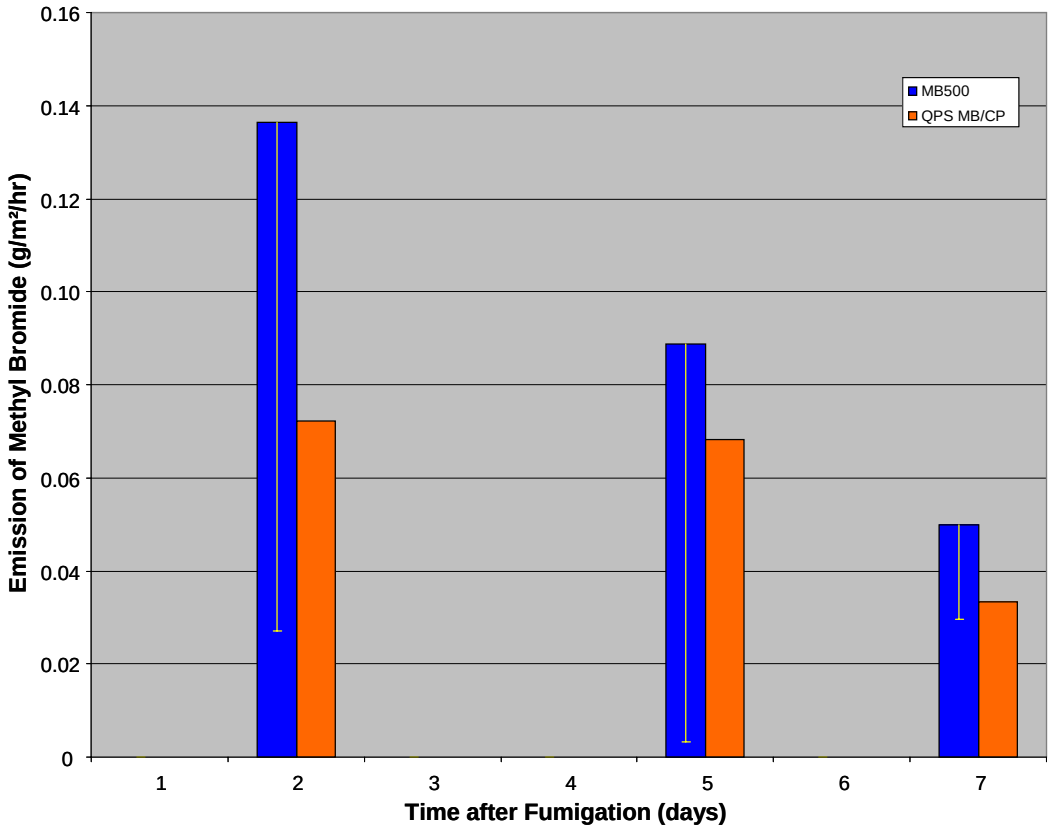


Figure 8.4. Emissions of methyl bromide from soil through low-density polyethylene film from two treatments in a field trial at Toolangi, Vic in 2009/10. Bars are LSDs where p = 0.05.

Table 8.12. Microbial activity (FDA hydrolysis) in soil (mg fluorescein/g soil/hr) over time following fumigation in a field trial at Toolangi. Vic in 2009/10. Values followed by different letters are significantly different, where p = 0.05.

Treatment	Time after fumigation				
	1 week	2 weeks	4 weeks	8 weeks	16 weeks
Untreated	0.610a	0.567a	0.763b	0.648a	0.620a
QPS MB	0.402b	0.291b	0.416b	0.439b	0.544ab
CP	0.320bc	0.355b	0.418b	0.401b	0.521bc
QPS MB/CP	0.257c	0.312b	0.381b	0.368bc	0.574ab
MI350	0.342bc	0.290b	0.490b	0.407bc	0.495bc
TC35	0.315bc	0.330b	0.399b	0.304c	0.419c
MB400	0.358b	0.381b	0.435b	0.372bc	0.500bc
MB500	0.260c	0.347b	0.438b	0.358bc	0.517b
LSD (p = 0.05)	0.095	0.127	0.149	0.124	0.095

Soil chemistry

There were no significant differences between treatments for any soil chemistry parameter tested (Table 8.13), at any sampling time. However, there was a trend towards higher ammonium levels and reduced nitrate levels in fumigated soils (Figure 8.5), which is consistent with the scientific literature (Porter et al., 2006).

Table 8.13. Chemical parameters of soils treated with various fumigants (4 weeks after treatment) in a field trial at Toolangi, Vic in 2009/10. Units are in ppm, except for pH. There was no significant difference (p = 0.05) between treatments.

Treatment	pH	NO_3	NH_4	P	K	S	Na	Ca	Mg	Cu	Zn	Fe	Mn	B
Untreated	5.92	16.2	1.4	38.6	0.75	2.6	0.34	8.8	1.4	1.4	0.65	96.0	21.7	0.22
QPS MB	5.65	13.2	3.9	39.7	0.78	3.4	0.41	7.3	1.4	1.9	0.77	96.0	20.2	0.32
CP	5.77	13.3	2.9	40.6	0.78	2.5	0.39	7.5	1.5	1.7	0.75	95.2	21.0	0.27
QPS MB/CP	5.65	13.2	3.9	39.7	0.78	3.4	0.41	7.3	1.4	1.9	0.77	96.0	20.2	0.32
MI350	5.90	11.3	1.9	39.3	0.83	2.5	0.41	7.4	1.3	1.6	0.70	86.7	22.2	0.25
TC35	5.85	11.6	2.4	40.3	0.83	2.6	0.44	6.8	1.2	2.0	0.75	96.5	22.2	0.27
MB400	5.85	9.8	9.6	41.8	0.78	2.4	0.38	7.3	1.2	1.5	0.67	99.0	23.5	0.25
MB500	6.07	10.3	4.2	40.6	0.85	2.5	0.4	8.1	1.2	1.7	0.82	96.0	21.7	0.22

Pythium concentration

All fumigants, except QPS MB, significantly reduced the concentration of *Pythium* spp. in soil compared with the untreated control (Table 8.14). QPS MB/CP reduced *Pythium* spp. by 92%, which was equivalent to the control by the fumigants MB500, MB400, MI350, and TC35.

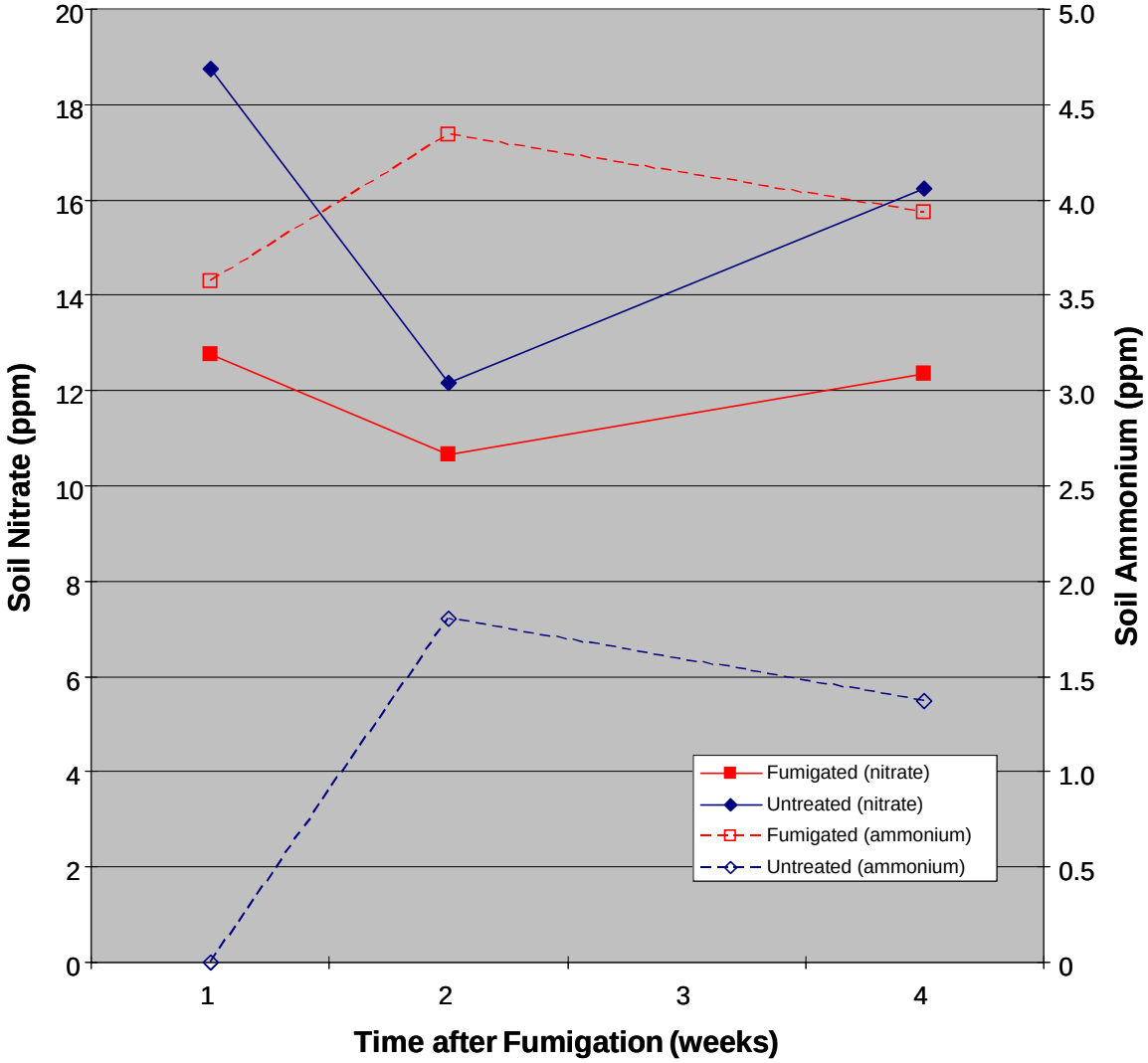


Figure 8.5. Trends in nitrate and ammonium concentrations between fumigated (average of all treatments) and untreated soils in a field trial at Toolangi, Vic. There was no significant (p = 0.05) difference between treatments.

Table 8.14. Average Pythium spp. (clade F) concentrations in soils treated with different fumigants in a field trial at Toolangi, Vic in 2009/10. Values followed by different letters are significantly different, where p = 0.05. Data was analysed following a $\log_{10}$ (x+0.1) transformation.

Fumigant	***Pythium* spp. Concentration (pg DNA/g soil)**
Untreated	74.6 a
QPS MB	38.8 a
CP	11.2 b
QPS MB/CP	6.4 b
MI350	6.0 b
TC35	10.3 b
MB400	8.8 b
MB500	4.4 b

Weed emergence

Eluesine indica (Indian goosegrass) (27%), *Chenopodium ambrosioides* (Mexican tea) (18%), and *Amaranthus retroflexus* (red-root amaranth) (18%) were the dominant weeds to emerge on the site. Treatment with all soil fumigants reduced weed emergence compared with the untreated control (Figure 8.6). QPS MB/CP reduced weed emergence (by 87% compared with the untreated control) to equivalent levels as MB500 and MI350. Co-application of QPS MB and CP significantly improved weed control compared with either component applied alone.

Runner yields

Runner yields in plots treated with QPS MB/CP were equivalent to those in MB500, MB400 and MI350-treated plots (Table 8.15). Furthermore, yields in the QPS MB/CP treatments were higher than in the untreated control (by 180%) and the TC35 treatment (by 60%). The low yields in TC35 plots were from crop phytotoxicity, which was most likely due to the long residual times of 1,3 dichloropropene in soil. Co-application of QPS MB with CP increased yields compared either treatment applied alone. There were no significant differences between the average crown diameters of harvested runners between treatments (Table 8.15).

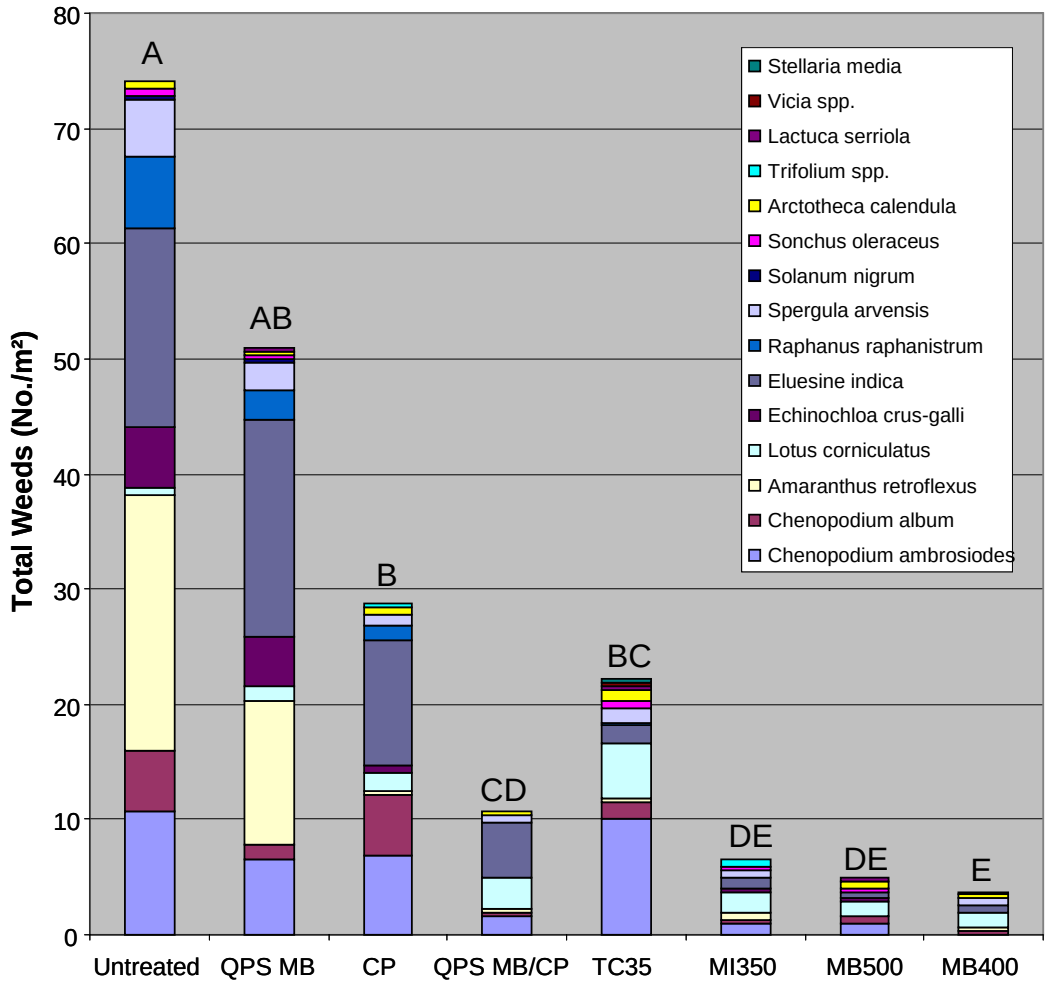


Figure 8.6. Weed emergence in soils treated with different fumigants in a strawberry field trial at Toolangi, Vic in 2009/10. Bars followed by different letters are significantly different, where p = 0.05. Analysis was made on $\log_{10}(x+1)$ transformed data.

Table 8.15. Final yields and crown diameters of strawberry runners grown in various fumigant treatments in a field trial at Toolangi, Vic in 2009/10.

Fumigant	Runner Yield (plantlets/m)	Crown Diameter (mm)
Untreated	25.8 c	15.26
QPS MB	59.8 ab	13.83
CP	40.0 bc	14.55
QPS MB/CP	72.2 a	13.93
MI350	74.5 a	14.33
TC35	45.0 b	14.33
MB400	63.5 a	14.65
MB500	71.8 a	13.13
LSD (p = 0.05)	18.12	ns

Black root score and crown rot incidence

Soil treatment with all fumigants reduced the severity of black roots in harvested runners (Table 8.16). QPS MB/CP reduced the severity of black roots in strawberry runners to equivalent levels as MB500, MB400, and MI350, and to higher levels than TC35. The incidence of crown rot caused by *Phytophthora cactorum* in the trial was low and could not be statistically analysed. However, the trend was towards lower incidence of crown rot in plants grown in fumigated soils.

Table 8.16. Black root score and crown rot incidence in strawberry runners grown in soils treated with various fumigants in a field trial at Toolangi, Vic in 2009/10.

Fumigant	Black Root Score (0-5)	Incidence of Crown Rot (%)
Untreated	1.92 a	0.125
QPS MB	0.72 c	0.000
CP	0.62 c	0.000
QPS MB/CP	0.50 c	0.000
MI350	0.67 c	0.000
TC35	1.35 b	0.025
MB400	1.00 bc	0.025
MB500	0.72 c	0.000
LSD (p = 0.05)	0.51	-

8.3.3 Trial 3 (2011/12)

Runner yield

Runner yields in plots treated with QPS MB and Dazomet were significantly higher than untreated plots (Table 8.3). The use of VIF with QPS MB increased runner yields at low application rates, compared with LDPE plots, but not at high rates. Results for crown diameter of harvested runners followed similar trends to yields (Table 8.17).

Table 8.17. Black root score and crown rot incidence in strawberry runners grown in soils treated with various fumigants in a field trial at Knoxfield, Vic in 2011-2012.

Treatment	Runner yield (plantlets/m)	Crown diameter (mm)
Untreated	56.80 c	13.25 c
Dazomet	153.50 a	15.65 b
QPS MB100	150.80 b	16.10 b
QPS MB75	170.50 a	16.03 b
QPS MB50	105.50 c	15.35 b
QPS MB25	94.80 c	15.40 b
VIF QPS MB100	149.00 b	19.78 a
VIF QPS MB75	138.50 b	16.95 b
VIF QPS MB50	152.00 ab	19.48 a
VIF QPS MB25	127.80 b	16.43 b
LSD (p=0.05)	19.22	2.15

Disease incidence

Dazomet and higher rates of QPS MB treatments were significantly better at controlling the severity of black roots on harvested runners than lower rates of QPS MB and the untreated control in harvested runners (Table 8.18). There was no effect of VIF film on black root severity.

Table 8.18. Black root score in strawberry runners grown in soils treated with various fumigants in a field trial at Knoxfield, Vic in 2011-2012.

Treatment	Black root Score (0-5)
Untreated	2.06 c
Dazomet	1.18 a
QPS MB100	1.15 a
QPS MB75	1.45 b
QPS MB50	1.43 ba
QPS MB25	1.58 b
VIF QPS MB100	1.18 a
VIF QPS MB75	1.30 ab
VIF QPS MB50	1.48 b
VIF QPS MB25	1.50 b
LSD (p=0.05)	0.16

8.4 Discussion

The strawberry runner industry currently relies on soil disinfestation with methyl bromide (an ozone depleting substance) to maintain domestic biosecurity and market access. Although methyl bromide is being phased-out for soil uses under the *Montreal Protocol*, quarantine uses remain non-regulated. Therefore, the strawberry runner industry could legitimately use recaptured methyl bromide from quarantine applications (QPS MB) for soil disinfestation. This would allow Australia to reduce imports and use of methyl bromide by 30 tonnes p.a. (the amount currently imported for the runner industry), and cease annual requests to the UN for critical-use-exemptions in the industry. Unlike other alternative fumigants, government and

industry authorities already accept the active ingredient (methyl bromide) in QPS MB for meeting certification and domestic market access standards for strawberry runners.

When co-applied with chloropicrin, our research showed that QPS MB provided equivalent efficacy to standard shank-injected formulations for soil disinfestation. In comparison with the untreated control, soil disinfestation with QPS MB co-applied with chloropicrin gave:

- 100% control of laboratory-grown sclerotia of the strawberry pathogens *Sclerotium rolfsii* and *Verticillium dahliae*,
- 92% control of *Pythium* spp. (including species pathogenic to strawberry) in soil,
- 87% control of emerging weeds,
- 180% increase in strawberry runner yields,
- accelerated microbial recolonisation of soils compared with traditional fumigants,
- a trend towards reduced emissions of methyl bromide to the atmosphere from treated soils compared with traditional formulations.

When applied without chloropicrin, QPS MB gave a soil disinfestation effect, but not to the same level as standard shank-injected formulations. In comparison with the untreated control, soil disinfestation with QPS MB gave:

- 100% control of artificial inoculum and laboratory-grown sclerotia of the strawberry pathogens *Phytophthora cactorum*, *Rhizoctonia fragariae*, *Sclerotium rolfsii*, and *Verticillium dahliae*,
- 48% - 67% control of *Pythium* spp. (including species pathogenic to strawberry) in soil,
- 32% - 79% control of weeds,
- 52% - 132% increase in strawberry runner yields.

Evidence also showed that increasing applications rates and sealing soils with low-permeability barrier films (VIF) increased the efficacy of QPS MB applied without chloropicrin for soil disinfestation.

In addition to providing an alternative to shank-injected methyl bromide, other potential benefits of using QPS MB for soil fumigation in the strawberry runner industry may include:

- Reduced emissions of methyl bromide from fumigated soils to the atmosphere, as the carbon in QPS MB may adsorb fumigant residues thereby allowing greater breakdown in soil.
- Increased carbon sequestration into soils in the runner industry, as the activated carbon in QPS MB is highly inert and resistant to biological breakdown. This may offer possible benefits under future carbon-reduction scenarios.
- Reduced impact on soil health following fumigation, as QPS MB increases total soil carbon and may accelerate recolonisation of soils by microflora.
- Increased incentives for QPS fumigators to adopt recapture systems for methyl bromide (i.e. carbon waste could be on-sold to the runner industry).
- Reduced waste disposal issues for QPS users of recapture systems for methyl bromide, as the runner industry could use carbon waste.

Currently QPS MB is not registered, and therefore not available for use in the strawberry runner industry. In addition to developing a registration package, the engineering of commercial application technologies that can apply the product over large areas is a key requirement for the further development and ultimate adoption of QPS MB by the runner industry.

9. Rate Reduction Strategies for MB (Activity 7)

9.1 Introduction

In addition to finding and adopting alternatives, the runner industry has prioritised recent research towards the evaluation of methods for reducing emissions of currently exempted methyl bromide (MB) from soil to the atmosphere. This work has focussed on minimising application rates and investigating the use of impermeable barrier films, which trap fumigants more effectively in soil that standard low density polyethylene (LDPE) films. In 2005-06, a small-scale trial conducted in the Victorian runner industry showed that MB:Pic (50:50) applied at 12.5g MB/m² controlled soil-borne pathogens and weeds, and produced equivalent crop yields to standard rates (25g MB/m²). However, the trial also showed that the use of impermeable barrier films (Bromostop®, Canslit® and Orgalloy®) did not allow reduced application rates of MB:Pic compared with standard LDPE films. This was mostly attributed to the short time that films can remain in place in the industry (c. 5-7 days). Moreover, impermeable films were shown to pose an increased off-gassing risk to operators, compared with LDPE, when they were removed (Mattner et al., 2008). For this reason, industry considered that lowering application rates of MB:Pic would have the greatest potential for minimising emissions of MB to the atmosphere from soil fumigation in the runner industry.

Following this research in 2009, the UN Methyl Bromide Technical Options Committee challenged the Australian runner industry to reduce application rates of MB:Pic from 25g MB/m² to 20g MB/m². Currently, however, lower rates of MB:Pic are not registered in Australia or Victoria, and growers cannot legally apply the formulation below a rate of 25g MB/m². To support the possible registration of lower rates of MB:Pic, at least two years of bioequivalency data is required from both small-scale and commercial-scale trials.

The aim of the trials described in this chapter was to evaluate, in scientific trials and commercial-scale applications, the efficacy of MB:Pic applied at 20 gMB/m² compared with 25 gMB/m² for pathogen and weed control and for runner yields.

9.2 Materials and methods

Eight commercial and small-scale trials investigating lower rates of MB:Pic for soil disinfestation in the strawberry runner industry were established on commercial strawberry runner farms in the Toolangi Plant Protection District between 2009 and 2012. Trial beds were prepared and maintained for strawberry runner production using standard industry practices. Trials were conducted on flat rows, which were broad-acre fumigated following normal soil preparation (rotary hoeing and incorporation of lime). Individual plots were between 25 - 100 m in length and 3 – 15 m in width. All fumigants were shank injected into soil to a depth of 20 cm through tynes spaced 20 cm apart using a commercial rig (R&R Fumigation Services), and the soil surface sealed with low density polyethylene (30 µm thickness). Fumigation occurred between March to June each year. Between 1-2 weeks after fumigation,

barrier film was removed and the soil allowed to air prior to planting. Plots were planted with strawberry mother plants 10-12 weeks after fumigation (August to September) spaced 50 to 70 cm apart. Strawberry runners were harvested seven to ten months after planting (from March to June), depending on the variety grown. In general, untreated controls could not be included in trials because they were on commercial farms.

Except where otherwise stated, data were analysed using ANOVA as performed on the GENSTAT v. 12 statistical package (Lawes Agricultural Trust, IACR Rothamsted). Homogeneity of variance was determined by examining plots of fitted values versus residuals, while histograms of residuals were used to assess normality of distribution. Data transformations were made where appropriate. Fischer's LSD test was used to identify significant differences (where $p \leq 0.05$ and 0.10) between treatment means.

9.3 Results

9.3.1 Trials conducted in 2009-10

In 2009-10, a small-scale trial (randomised complete block design with four replicates) evaluated the effects of MB:Pic (40:60) at 600 kg/ha (20 gMB/m²), MB:Pic (50:50) at 400 kg/ha (20 gMB/m²), and MB:Pic (50:50) at 500 kg/ha (25 gMB/m²). The results showed similar effects of treatments on pathogen control and weed emergence, but the yield from plots treated with MB:Pic (50:50) at 400 kg/ha were reduced by 25% compared with runners in plots treated with the standard MB rate. Details are as follows:

- MB:Pic (40:60) at 600 kg/ha and MB:Pic (50:50) at 400 kg/ha (i.e. 20 gMB/m²) controlled pathogens (DNA concentrations of *R.solani* and *Pythium* spp. clade F, Figure 9.1) and weeds to the same level as MB:Pic (50:50) at 500 kg/ha (i.e. 25 gMB/m²).

- Runners (variety Gaviota) grown in plots treated with MB:Pic (40:60) applied at 600 $kg.ha^{-1}$ (i.e. 20 gMB/m²) produced the same yields as those in plots treated with MB:Pic (50:50) applied at 500 $kg.ha^{-1}$ (i.e. 25 gMB/m²) (Figure 9.2).

- Yields in plots treated with MB:Pic (50:50) applied at 400 kg/ha (i.e. 20 gMB/m²) were 25% lower than those in plots treated with MB:Pic (50:50) applied at 500 kg/ha^{-1} (i.e. 25 gMB/m²). This difference, however, was only significant at the $p = 0.10$ level of significance, but not at the $p = 0.05$ level (Figure 9.2).

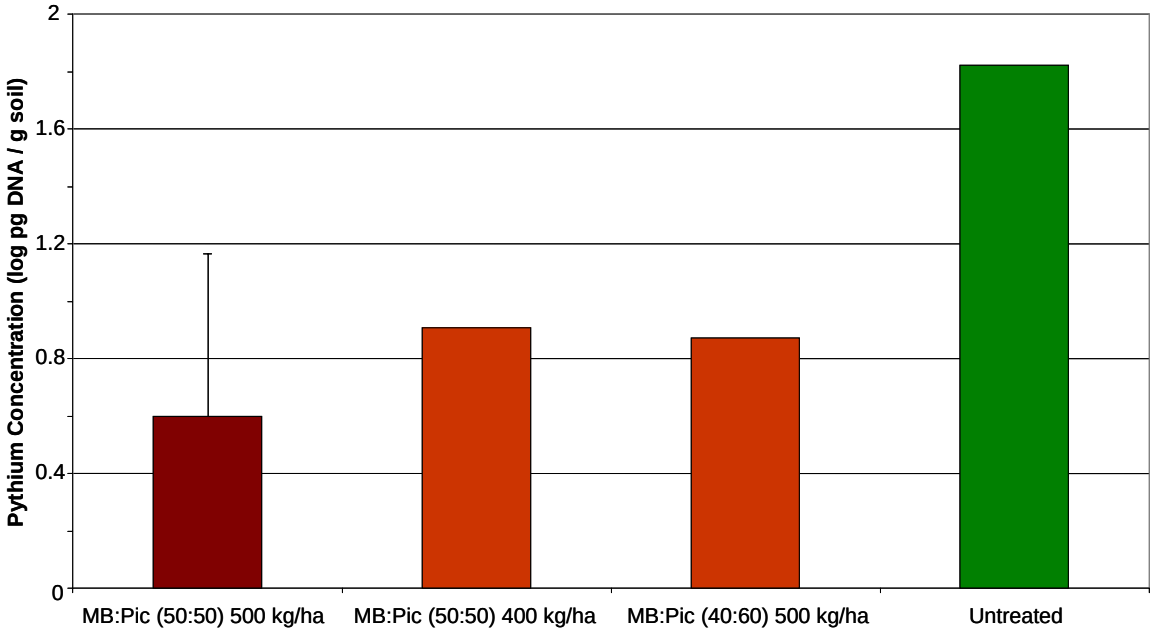


Figure 9.1. Concentration of DNA of *Pythium* clade F in soil four weeks after treatment with different rates and formulations of MB:Pic in a small-plot trial conducted at Toolangi Victoria in 2009-10. The bar is the least significant difference where p = 0.05.

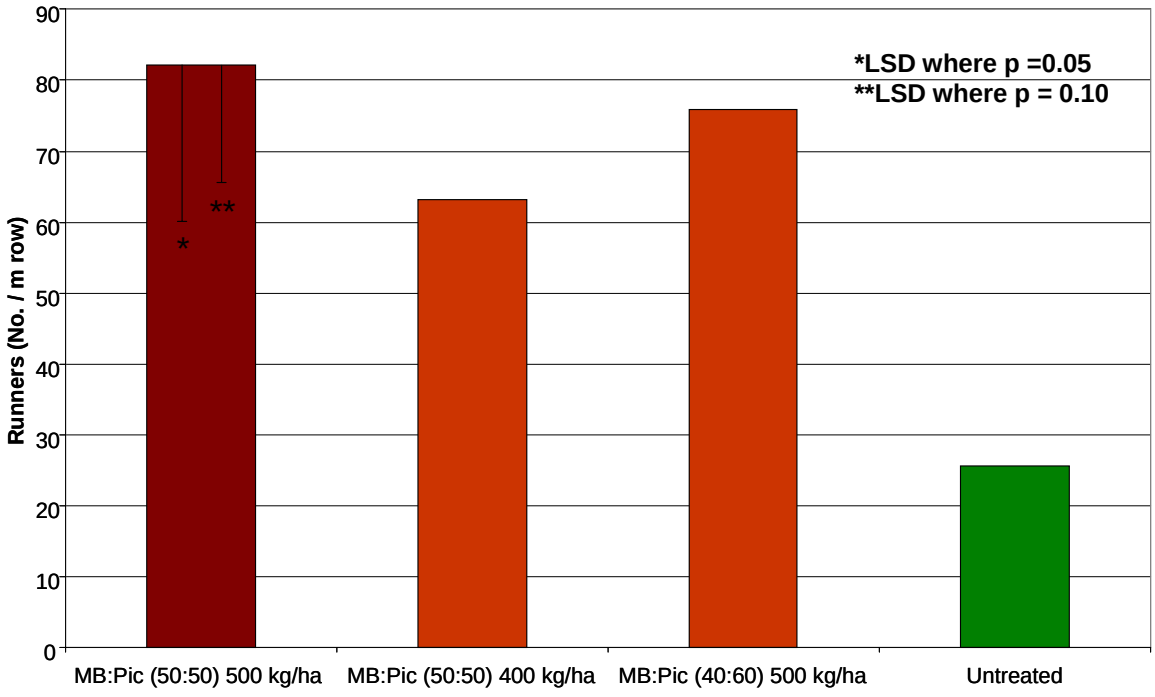


Figure 9.2. Runner yields of strawberries (var. Gaviota) grown in soils treated with different rates and formulations of MB:Pic in a small-plot trial conducted at Toolangi Victoria in 2009-10. The bars are the least significant differences where p = 0.05 and 0.10.

Three commercial-scale trials (randomised complete block designs with three replicates) of lower rate MB (MB:Pic 50:50 at 400 kg/ha, 20 gMB/m²), compared with the standard rate (MB:Pic 50:50 at 500 kg/ha, 20 gMB/m²), were also conducted in 2009-10. Results are summarised below:

- Weed control was significantly less with the lower rate of MB than with standard rate in two of the three trials (Figure 9.3).
- Pathogen control (concentration of DNA of *Pythium* Clade F in soil after treatment) was significantly less with lower rates of MB than standard rates in one of three trials (Figure 9.4).
- Runner yields were equivalent in plots treated with either rate of MB in all trials. Varieties tested in the trials included Festival, Aromas and Gaviota.

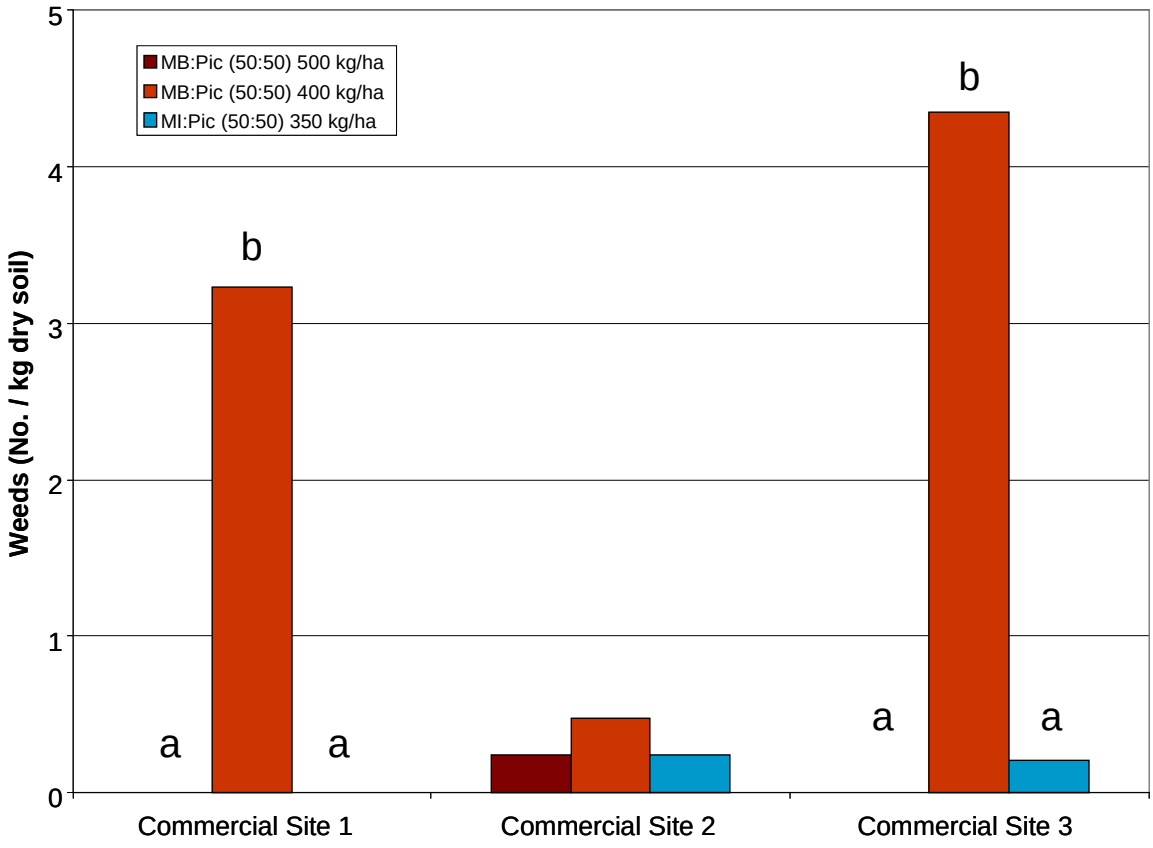


Figure 9.3. Weed emergence in soils treated with different fumigants in commercial-scale trials at Toolangi, Victoria in 2009-10. Bars followed by different letters at each commercial site are significantly different where $p \leq 0.05$.

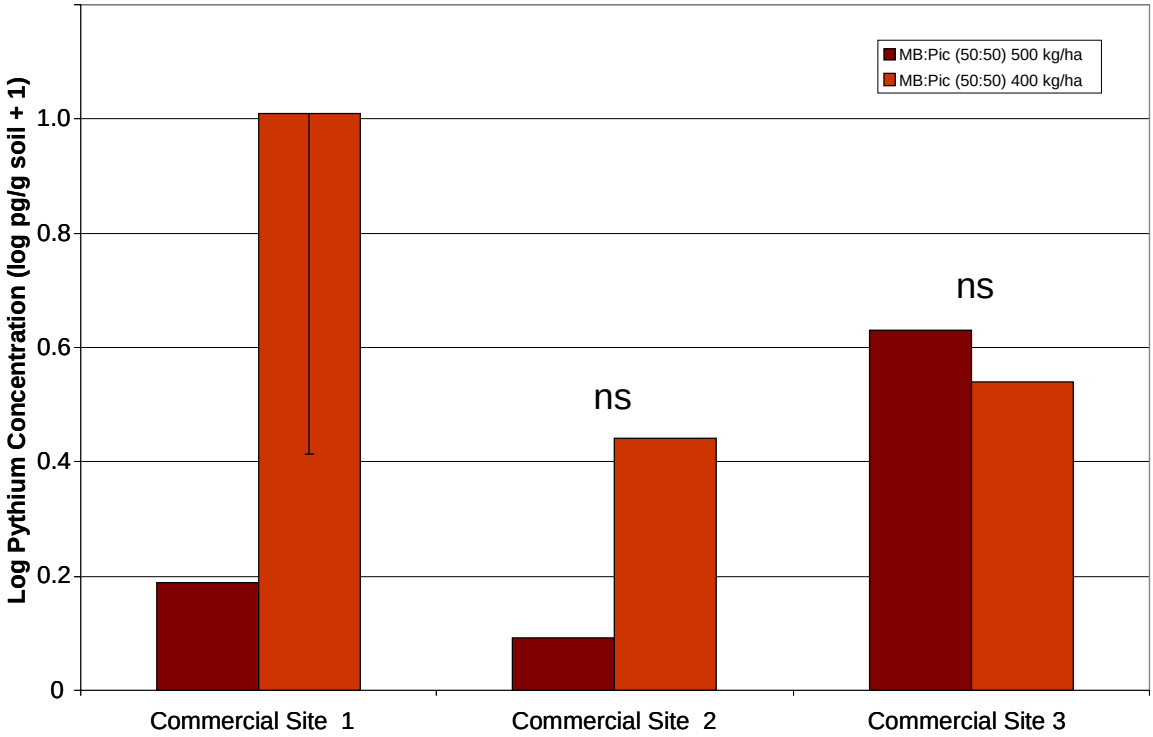


Figure 9.4. Concentration of DNA of *Pythium* clade F in soil four weeks after treatment with different rates of MB:Pic in commercial scale trials conducted at Toolangi Victoria in 2009-10. The bar is the least significant difference where p = 0.05 for Commercial Site 1 only.

9.3.2 Trials conducted in 2010-11

Three commercial-scale trials (randomised complete block designs with three replicates) of lower rate MB (MB:Pic 50:50 at 400 kg/ha, 20 gMB/m²), compared with the standard rate (MB:Pic 50:50 at 500 kg/ha, 20 gMB/m²), were conducted in 2010-11. Results are summarised below:

- There was a trend towards greater weed emergence (over double) in soil treated with the lower rate of MB than with the standard rate (Table 9.1). Weed control was significantly less with the lower rate of MB than with the standard rate in one of the three trials at the $p \leq 0.05$ level, and two of the three trials at the $p \leq 0.10$ level.

- There was a trend towards higher concentrations of *Pythium* clade F (8-fold higher) in soils treated with the lower rate of MB than with the standard rate (Table 9.2). *Pythium* control was significantly poorer in soil treated with lower rate MB than the standard rate in one of the three trials.

- Yields were equivalent in plots treated with either rate of MB in all trials. Varieties tested in the trials included Festival, Albion, and San Andreas.

Table 9.1. Weed emergence (weeds/m²), 1-month after planting, in three commercial trials investigating the effect of MB rate in the strawberry runner industry at Toolangi, Victoria in 2010/11.

Treatment	Trial A (var. Festival)	Trial B (var. San Andreas)	Trial C (var. Albion)	Average
MB:Pic 50:50 500 kg.ha^{-1}	5.9	5.9	4.4	5.4
MB:Pic 50:50 400 kg.ha^{-1}	10.6	14.7	9.4	11.6
LSD (p =0.05)	ns	6.8	ns	
LSD (p = 010)	4.3	-	ns	

Table 9.2. Concentrations of Pythium spp. in soil (pg DNA/g soil), at planting, in three commercial trials investigating the effect of MB rate in the strawberry runner industry at Toolangi, Victoria in 2010/11.

Treatment	Trial A (var. Festival)	Trial B (var. San Andreas)	Trial C (var. Albion)	Average
MB:Pic 50:50 500 kg/ha	1.0	2.1	14.0	5.7
MB:Pic 50:50 400 kg/ha	6.5	7.4	132.0	48.6
LSD (p =0.05)	3.7	ns	ns	
LSD (p = 010)	-	ns	ns	

9.3.3 Small plot trial conducted in 2011-12

In 2011-12 a small-plot trial was conducted comparing the efficacy of lower rate MB (MB:Pic 50:50 at 400 kg/ha, 20 gMB/m²) with the standard rate (MB:Pic 50:50 at 500 kg/ha, 20 gMB/m²). The trial was conducted as a randomised split-plot design with five blocks. Fumigant treatments formed the main plots and strawberry variety treatments (var. Albion and Festival) formed the split plots. Results are summarised below:

- Both rates of MB:Pic reduced inoculum levels of *Pythium* clade F to equivalent levels at a soil depth of 10 cm. However, the lower rate of MB:Pic had no impact on inoculum levels at 30-90 cm compared with the untreated control. By comparison, the standard rate of MB:Pic significantly reduced inoculum levels at depths of 30-90 cm compared with the control (Table 9.3).

- Both rates of MB:Pic reduced weed emergence compared with the control. However, weed emergence was four times higher in plots treated with the lower rate MB:Pic than the standard rate (Table 9.4).

Table 9.3. Concentrations of *Pythium* clade F (log_{10}(pg DNA / g soil + 1)) in soils treated with different rates of MB:Pic at three depths in a replicated trial at Toolangi, Vic (four weeks after treatment).

Treatment	**Soil Depth**		
	10 cm	**30 cm**	**90 cm**
MB:Pic 50:50 400 kg/ha	0.4	1.8	1.3
MB:Pic 50:50 500 kg/ha	0.2	1.2	0.6
Untreated	1.9	2.0	1.5
LSD (p = 0.05)*	0.3		

Table 9.4. Weed emergence (sqrt weeds / m²) in soils treated with different rates of MB:Pic in a replicated trial at Toolangi, Vic.

Treatment	**Weed Emergence**
MB:Pic 50:50 400 kg/ha	4.07
MB:Pic 50:50 500 kg/ha	1.90
Untreated	7.25
LSD (p = 0.05)*	1.00

9.4 Discussion

The results from trials demonstrate that the lower rate of MB:Pic (20 g MB/m²) does not provide equivalent weed control or pathogen control at depth as the current registered rate of MB:Pic (25 g MB/m²). Currently, chemical registration authorities do not accept this data as demonstration of bioequivalency, and lower rates of MB remain unavailable for use by runner growers. Furthermore, runner certification authorities consider that soil fumigation with lower rates of MB:Pic poses too high a biosecurity risk to approve its use. This risk, however, may need to be balanced against the environmental cost of the continued use of higher rates of MB. Future research should be directed towards better understanding the environmental factors that affect fumigation with lower rates of MB:Pic (most of the failures with the lower rates in the current trials occurred later in the season when conditions were becoming marginal). For example, it may be possible to fumigate with lower rates of MB:Pic when conditions are ideal earlier in the fumigation season and only use higher rates under more marginal conditions.

10. Reducing the Spread of Gnomoniopsis (Activity 8)

10.1 Introduction

The diseases leaf blotch, stem-end fruit rot and root rot in strawberry plants are caused by *Gnomoniopsis* spp. (syn. *Gnomonia*). The fungus, *Gnomoniopsis fructicola*, is an endophytic resident in strawberry plants and a weak pathogen of runner crops, but under optimal conditions for development it can cause economic losses in the strawberry fruit industry. In 2009, severe epidemics of stem-end rot disease caused strawberry fruit losses of 17% in the Queensland fruit industry (valued at $30 million). Circumstantial evidence suggests that the source of infection may have been runners harvested from infected runner crops. Currently, preventative control measures for *Gnomoniopsis* in the runner industry include long rotations with cover crops (such as rye corn and millet), fumigation with MB/Pic to kill inoculum surviving in crop debris, and fungicidal sprays with prochloraz. The key reasons influencing the development of recent epidemics of *Gnomoniopsis* are unknown but could include fungicide failures, favourable environmental conditions and/or poor management practices. Recent research has shown that improved hygiene practices (e.g. removal of older infected leaves after planting) and newer fungicide chemistries (e.g. some strobilurins) offer an alternative to prochloraz for managing *Gnomoniopsis* in runner production.

A series of laboratory, glasshouse and field studies were conducted to investigate the environmental factors influencing disease development, to screen new fungicides to compliment prochloraz for managing *Gnomoniopsis* in runner production and to monitor *Gnomoniopsis* incidence in the runner multiplication generations (see Chapter 5).

The aims of the research were to (1) investigate the influence of environmental conditions, temperature, humidity and rain events on infection and development of leaf blotch disease in strawberry runners; (2) investigate the efficacy of new fungicide formulations against *Gnomoniopsis* and residual effect of these chemistries in consecutive generations in the runner multiplication scheme; (3) develop an integrated fungicidal spray and hygiene program for growers to minimise the risk of fungicidal resistance developing in *Gnomoniopsis* populations; and (4) screen *Gnomoniopsis* incidence in the runner multiplication scheme in order to establish disease threshold (Chapter 5).

10.2 Materials and methods

10.2.1 Environmental study

Two inoculation tests and one controlled environmental study were carried out in 2010-2011 at DPI, Knoxfield. All test plants (tissue culture germplasm) were obtained from the DPI nucleus nursery and runner tips were grown to 3-4 leaf stage before use. The isolate of *G. fructicola* used in this study was derived from strawberry runners cv. Festival sampled from a grower's property at Toolangi, Victoria. Single spore culture of *G. fructicola* was prepared from these isolates and used in all *in-vitro* studies.

The experiment was conducted using a fully randomised experimental design. Treatments factors were inoculation at two levels (inoculated and non-inoculated); three temperature regimes (10ºC, 16ºC, 26ºC) and three temperature transition treatments (units from 10ºC were transferred to 16º C & 26ºC, and units from 16º C were transferred to 26ºC). Plants in the inoculated treatment were sprayed to run-off with a spore suspension (conidia) of 10^6/ml, while plants in the non-inoculated treatment were sprayed with sterile distilled water. Each treatment was replicated three times with three plantlets per treatment.

The experiment was run in three phases. Inoculated plantlets were placed in propagation trays and kept in the glasshouse under natural light and exposed to ambient photoperiods at temperatures of min 19.5ºC-max 21.3ºC and at 63% relative humidity which represented the first phase of the experiment (Table 10.1). Propagation trays containing the plantlets were moved into three environment controlled cabinets (ECC) which were set at 10, 16, or 26ºC. All cabinets were set at 80% relative humidity with a 12 h photoperiod (Table 10.2). Plantlets were allowed to grow in the cabinets for a further 6 days, which represented the second phase of the experiment (Table 10.1).

Following the second phase of the experiment, some plants in the 10ºC were moved to 16ºC and 26ºC cabinets and some plants in the 16ºC were moved to the 26ºC cabinet. Plantlets were allowed to grow for a further 7 days, which represented the third phase of the experiment (Table 10.1). Assessments for disease incidence (percentage of infected leaves) were carried out daily for the duration of the experiment. Disease severity (percentage of infected leaf tissue determined by image analysis) was assessed on detached leaves from all infected and control treatments at 14 days after inoculation. Recovery of *G. fructicola* was assessed from plantlets in all treatments to determine colonisation and pathogenicity.

Table 10.1. Design of the environmental experiment showing temperature treatments the inoculated runners were exposed to during incubation, infection and disease development phases in the glasshouse and environment controlled cabinets (ECC).

Treatments	**Inoc**	**Phase 1. Incubation (24 hours)**	**Phase 2. Disease infection and development (6 days)**			**Phase 3. Disease infection and development (7 days onwards)**		
		Glasshouse	ECC 10 ºC	ECC 16 ºC	ECC 26ºC	ECC 10 ºC	ECC 16 ºC	ECC 26 ºC
Control	No	*	*	*	*	*	*	*
10 ºC Control	Yes	*	*			*		
10 ºC - 16 ºC	Yes	*	*				*	
10 ºC -26 ºC	Yes	*	*					*
16 ºC Control	No	*		*			*	
16 ºC	Yes	*		*			*	
16 ºC -26 ºC	Yes	*		*				*
26ºC	Yes	*			*			*
26 ºC Control	No	*			*			*

Table 10.2. Temperature and relative humidity readings taken from data loggers (Tinytag®) kept inside propagation trays in ECC and glasshouse during experimentation. Data was collected and interpreted using Tinytag® software.

Environment	**Temperature (ºC)**			**Relative humidity (%)**		
Equipment	Mean	Max	Min	Mean	Max	Min
Low – ECC 10 ºC	9.9	11.3	8.5	89.0	96	82
Medium- ECC 16 ºC	15.9	16.5	15.9	82.9	85.1	80.7
High- ECC 26 ºC	25.6	26.8	24.4	89.8	96.2	83.4
Glasshouse	20.5	21.3	19.5	32.6	63.8	45.7

10.2.2 Fungicide *in-vitro* screening studies

Three *in-vitro* efficacy tests were carried out against *Gnomoniopsis* with five test fungicide formulations. The test formulations were selected from past studies conducted by interstate and overseas researchers on the control of foliar diseases of strawberry plants. None of these formulations were tested against the *Gnomoniopsis fructicola* isolates from strawberry varieties grown in Victorian strawberry nurseries. For *in-vitro* tests, the same single spore isolate of *G. fructicola* was used. Both

vegetative (hyphal) and reproductive (spore) phases of the organism were used in the tests. The fungicides were tested for their fungistatic and fungicidal properties. Petri plate assays were used and the radial growth of four fungal colonies measured to determine the effect of the fungicides.

Three *in-vitro* Petri plate assays (one disk diffusion bioassay, and two agar amendment bioassays) were carried out to screen five test fungicide formulations against *G. fructicola* (Table 10.3). In the first two assays the fungicides were tested against spores (conidia) and mycelium (disk diffusion and agar amendment bioassay), and the third assay tested the fungicides against mycelium only (agar amendment bioassay). Prochloraz (a fungicide known to be effective against *G. fructicola*) was used in all experiments as a positive control. The fungicide concentrations used in the bioassays were equivalent to the recommended application rate of the product (Table 10.3). There were 10 replicates of each treatment in the assays. All tests were carried out on a laboratory bench under ambient temperature and light conditions. Data were analysed using Genstat 13.0 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK) ANOVA procedures with means being compared using Fisher's Protected Least Significance Differences (l.s.d.) test at the 0.05 probability level. Where data showed heterogeneous variance (even after data transformation), means were compared by standard error.

Table 10.3. Fungicide formulations used in both the in-vitro and field studies. Note: Not all of these formulations are registered for use in strawberry runner production.

Fungicide	Group	Active ingredient(s)	Recommended Label Rates
Octave ®	3	Prochloraz	100g/100L
Amistar Top®	11&3	Azoxystrobin, Difenoconazole	40ml/100L
Cabrio ®	11	Pyraclostrobin	40ml/100L
Flint®	11	Trifloxystrobin	25g/100L
Pristine®	7&11	Boscalid, Pyraclostrobin	40g/100L
Switch®	9 &12	Cyprodinil, Fludioxonil	80g/100L

10.2.3 Field trials

Field trials were conducted from 2009-2012 on commercial runner blocks (200 m x 200 m) at Toolangi, Victoria (37° 32' 0" South, 145° 28' 0" East) on a kraznosem soil (silty clay). Soils were fumigated with a mixture of methyl bromide and chloropicrin (50:50, 500 kg/ha) in May prior to planting. Approximately twenty weeks after fumigation (September), the trial blocks were transplanted with strawberry runners spaced 1 m apart in rows spaced 2.7 m apart. Two strawberry varieties, Festival and Albion, were used in all the field trials. Albion proved less susceptible to *G. fructicola* than Festival, and therefore results are presented for Festival plants only. Trial plots were prepared and maintained using standard industry practices, except that the growers only sprayed for insect pests not for disease. Individual plots comprised of five mother plants, which were replicated four times. Fungicides were applied commencing from four weeks after planting when runners had reached the 2-3 leaf stage. Two disease assessments were carried out, one before the first fungicide

application and a second at harvest. Strawberry runners were harvested seven to ten months after planting. Runner yield (number of runners per square metre) and crown diameter were measured at harvest. Except where otherwise stated, data were analysed using ANOVA as performed on the GENSTAT v. 12 statistical packages (Lawes Agricultural Trust, IACR Rothamsted). Fischer's LSD test was used to identify significant differences ($p \leq 0.05$) between treatment means.

Prochloraz application trial

The season for strawberry runner production was divided into three growth periods (GP): early period (Oct-Dec) –mother plant establishment; middle period (Dec-Feb) – runner development; and late period (Feb-Apr) - runner maturity. Prochloraz was applied every four weeks to GP treatments (Table 10.4) except for positive control (PC) where it was applied every fortnight throughout the season (current recommendation for treating *G. fructicola* in runners). The negative control was sprayed with water only, every fortnight throughout the season. The incidence of leaf blotch and spot (% of affected leaves per mother plant) was conducted at harvest. Petioles (20) were sampled near harvest and the incidence (%) of *G. fructicola* infection determined using cultural techniques.

Runners harvested from the treatments in the above trial were transplanted onto commercial fruit farms in Queensland and Victoria. Normal grower practices were followed in establishing and maintaining the fruit trials. Incidence of *Gnomoniopsis* was determined by carrying out isolations from petioles samples (20) from month old plants from each of the treatments. The incidence of stem end rot (% of infected fruit+calyx) was determined at first commercial fruit pick, midway through the season, and at the end of the season.

Table 10.4. Prochloraz application treatments imposed during various growth periods (GP) of runner production to determine the optimum application schedule for the control of leaf blotch (caused by *G. fructicola*) in strawberry runners.

	2009			**2010**			
Growth periods	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**
Early (EGP)	*	*	*				
Mid (MGP)			*	*	*		
Late (LGP)					*	*	*
Pos Ctrl (PC)	* *	* *	* *	* *	* *	* *	* *
Neg Ctrl (NC)	O	O	O	O	O	O	O
* Fungicide applied once per month * * Fungicide applied every two weeks O fungicide not applied at all							

Prochloraz alternatives trial

The test fungicides examined in the in-vitro screening studies (Section 10.2.2) were examined for their efficacy in controlling *G. fructicola* and leaf blotch in strawberry runners in the field. Fungicides were applied fortnightly throughout the growing seasons at the rates listed in Table 10.3. A sequential rotation of the test fungicides (Azoxystrobin + Difenoconazole; Pyraclostrobin; Cyprodinil + Fludioxonil; Trifloxystrobin; Boscalid + Pyraclostrobin) was also included as one treatment in the trial. The positive control was Prochloraz and the negative control was sprayed with water only. The trial was conducted over two consecutive seasons (2010/11 and

2011/12) in the mother and then the commercial generation of the runner scheme. Here, runners from the mother plant generation were harvested and planted in the commercial generation where the same treatments were applied. The incidence of leaf blotch and *Gnomoniopsis* was monitored and runner yields taken at harvest.

Spray program trial

Two fungicide programs (P1 & P2) were investigated for their efficacy against leaf blotch and *Gnomoniopsis* in this trial. Each of the programs had two formulations from two different fungicide groups (Table 10.5). P1 contained the compatible fungicides Boscalid + Pyraclostrobin and Prochloraz, while P2 contained Cyprodinil + Fludioxonil and Azoxystrobin + Difenoconazole. Both the programs consisted of three fortnightly applications made throughout the growing season with a two-week break (no formulation applied) between them (Table 10.5). The individual components of the spray programs were included as controls in the trial, in addition to the industry standard (Prochloraz) and an untreated control. Incidence of leaf blotch and *Gnomoniopsis* was assessed on mother plants at stolon initiation and before harvest.

10.3 Results

10.3.1 Environmental study

Infection and development of symptoms in inoculated plantlets

Phase 1: No symptoms appeared on plantlets in phase 1 of the experiment when the plants were kept in the glasshouse for 24 hrs.

Phase 2: Symptoms of dark spots first appeared in plantlets maintained at 26ºC. By the end of phase 2, disease incidence in plantlets at 26ºC was 60% (Figure 10.1). Disease symptoms did not appear in plantlets kept at lower temperature regimes (10-16ºC) during phase 2 (Figure 10.1).

Phase 3: Symptoms were starting to appear in plantlets that were kept constantly at 16ºC and plantlets that were moved from 10ºC and 16ºC to 25ºC. By day 14, disease incidence in these treatments reached 60%, compared with 80% for the plantlets that were kept constantly at 26ºC for the same time. None of the plants in the other temperature treatments showed any symptoms of the disease at 14 days. Plantlets moved from 10 to 26ºC had significantly higher disease severity compared with all other treatments. Even though treatment 26ºC had higher disease incidence it did not have a higher severity than other treatments (Table 10.6).

Table 10.5 Spray programs investigated in a field trial at Toolangi, Victoria in 2011/12.

Fungicide programs	**Oct**		**Nov**		**Dec**		**Jan**		**Feb**		**Mar**		**Apr**	
Unsprayed *(Neg)*	**o**	**o**	**o**	**o**	**o**	**o**	**o**	**o**	**o**	**o**	**o**	**o**	**o**	**o**
Prochloraz *(Pos)*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	Program 1													
Prochloraz (2 sprays)	*	*				*	*				*	*		
Boscalid pyraclostrobin (1spray)			*					*					*	
Prochloraz (Control)	*	*				*	*				*	*		
Boscalid Pyraclostrobin (Control)			*					*					*	
	Program 2													
Azoxystrobin Difenoconazole (2 sprays)	*	*				*	*				*	*		
Cyprodinil Fludioxonil (1 spray)			*					*					*	
Azoxystrobin Difenoconazole (Control)	*	*				*	*				*	*		
Cyprodinil Fludioxonil (Control)			*					*					*	

* -Fungicide applied once per month
O -Fungicide not applied at all

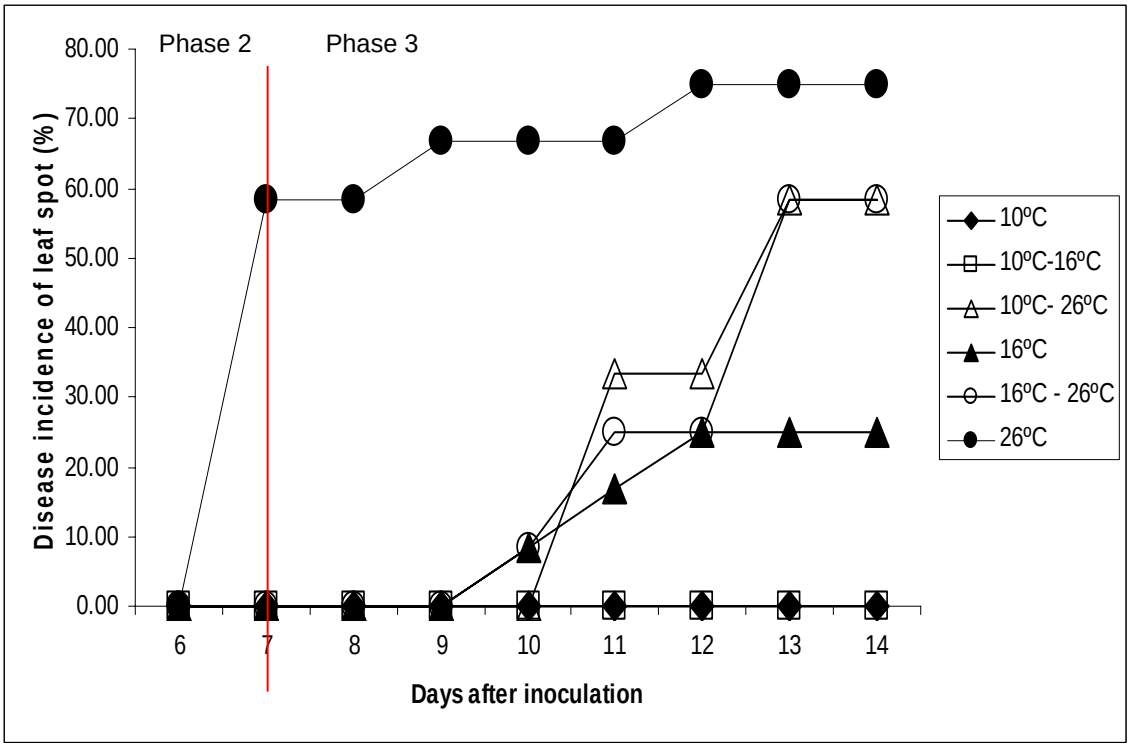


Fig. 10.1. Progress of leaf spot incidence (% of disease leaves) in plantlets inoculated with spore suspension of *G. fructicola* and kept at various temperature treatments in environment controlled cabinets (ECC) (RH 80%). Uninoculated control is not plotted because there was no disease present. All the treatments with solid point markers were not moved from original ECC in phase 3 of the experiment.

Table 10.6. Percentage disease incidence and severity of dark spots on leaves of plantlets inoculated with spore suspension of *G. fructicola*. Disease incidence and severity was assessed at day 14 after inoculation.

Treatment	Disease incidence (%)	Disease severity (%)
10ºC (phase 2) – 26ºC (phase 3)	58.3 b	1.8 b
16ºC (phase 2 & 3)	25.0 a	1.4 ab
16ºC (phase 2) – 26ºC (phase 3)	58.3 b	0.94 a
26ºC (phase 2 & 3)	75.0 c	1.1 a
l.s.d. (5%)	12.1	0.56

Recovery and pathogenicity of Gnomoniopsis

Gnomoniopsis fructicola was recovered from all inoculated plantlets with or without disease incidence. No fungus was isolated from any of the uninoculated treatments (Table 10.7). Pycnidia and perithecia were produced in petiole tissue and culture plates.

Table 10.7. Recovery of *G. fructicola* from inoculated and uninoculated plantlets at completion of experiment. Isolations were carried out from asymptomatic leaves and petioles where possible.

Treatment	No. of Leaves sampled (%)a	No. of Petiole sampled (%)a	Disease incidence
10ºCcontrol	6 (0.0)	6 (0.0)	No
10ºC	6 (100.0)	6 (100.0)	No
10ºC-16ºC	6 (100.0)	6 (100.0)	Yes
10ºC- 26ºC	6 (100.0)	6 (100.0)	Yes
16ºC control	6 (0.0)	6 (0.0)	No
16ºC	6 (100.0)	6 (100.0)	Yes
16ºC - 26ºC	6 (100.0)	6 (100.0)	Yes
26ºC control	6 (0.0)	6 (0.0)	No
26ºC	6 (100.0)	6 (100.0)	Yes

a: Number of leaves and petioles resulting in re-isolation of *G. fructicola*, percentage in bracket

10.3.2 Fungicide *in-vitro* screening studies

In a disk diffusion bioassay, the positive control (Prochloraz) inhibited fungal growth significantly more than all other test fungicides and the negative control (untreated) (Table 10.8). However, Azoxystrobin + Difenoconazole also showed strong inhibition of fungal growth.

Table 10.8. Mean % inhibition (compared with the untreated control) of growth of *G. fructicola* by fungicides in a disk diffusion assay. Mean values followed by different letters in each column are significantly different, $p \leq 0.05$.

Treatment	Inhibition of fungal growth (%)
Prochloraz (positive control)	81.26 a
Azoxystrobin + Difenoconazole	73.57 b
Pyraclostrobin	26.40 c
Trifloxystrobin	22.70 c d
Boscalid + Pyraclostrobin	19.57 d
Cyprodinil + Fludioxonil	8.86 e
Untreated (negative control)	0.00 f
l.s.d (0.05)	4.48

All fungicides significantly inhibited fungal growth compared with the untreated control when spores of the organism were exposed to the fungicides in an amended agar bioassay (Table 10.9).

Table 10.9. Inhibition of fungal growth from conidia of *G. fructicola* by fungicides in an amended agar assay. Mean values followed by different letters are significantly different, $p \leq 0.05$.

Treatment	Inhibition of fungal growth (%)
Prochloraz (positive control)	100.0 a
Azoxystrobin + Difenoconazole	100.0 a
Pyraclostrobin	100.0 a
Trifloxystrobin	63.4 (SE*=6.4) b
Boscalid + Pyraclostrobin	100.0 a
Cyprodinil + Fludioxonil	100.0 a
Untreated (negative control)	0.00 c

SE= Standard error

Only trifloxystrobin inhibited growth significantly less than the positive control (prochloraz) when fungicides were tested against the mycelial growth of G. fructicola in an amended agar bioassay (Table 10.10). Of all the fungicide treatments, only Prochloraz and Azoxystrobin + Difenoconazole had a fungicidal (rather than fungistatic) effect on the mycelium of the fungus (Table 10.10).

Table 10.10. Inhibition of fungal growth arising from a mycelium plug of *G. fructicola* by fungicides in an amended agar assay, followed by a test to determine whether the treatments were fungicidal or fungistatic. Mean values followed by different letters in each column are significantly different, $p \leq 0.05$.

Treatment	Inhibition of fungal growth (%)	Activity
Prochloraz (positive control)	100.00 a	Fungicidal
Azoxystrobin+ Difenoconazole	100.00 a	Fungicidal
Pyraclostrobin	100.00 a	Fungistatic
Trifloxystrobin	87.0 (SE*= 6.4) b	Fungistatic
Boscalid + Pyraclostrobin	100.00 a	Fungistatic
Cyprodinil + Fludioxonil	100.00 a	Fungistatic
Untreated (negative control)	0.00 c	Fungistatic

* SE= standard error

10.3.3 Field trials

Prochloraz application trial

Fortnightly application of Prochloraz (positive control) significantly reduced the incidence of leaf blotch and spot in runners compared with the negative control, and monthly application treatments. Monthly applications during the mid growth period (MGP) was more effective than application during the early growth period (EGP) in reducing leaf blotch incidence (Figure 10.2). When runners from these treatments were checked for *Gnomoniopsis*, a similar trend was observed (Figure 10.3). Runner yields for all the fungicide treatments were the same (data not shown). It was evident that Prochloraz was effective against *Gnomoniopsis* but there was no evidence that there is an association between leaf blotch incidence and runner yield. *Gnomoniopsis* has an endophytic association with strawberries and can behave as a latent pathogen for leaf blotch and spot disease, but this association is yet to be related to stem-end rot disease of strawberry fruit.

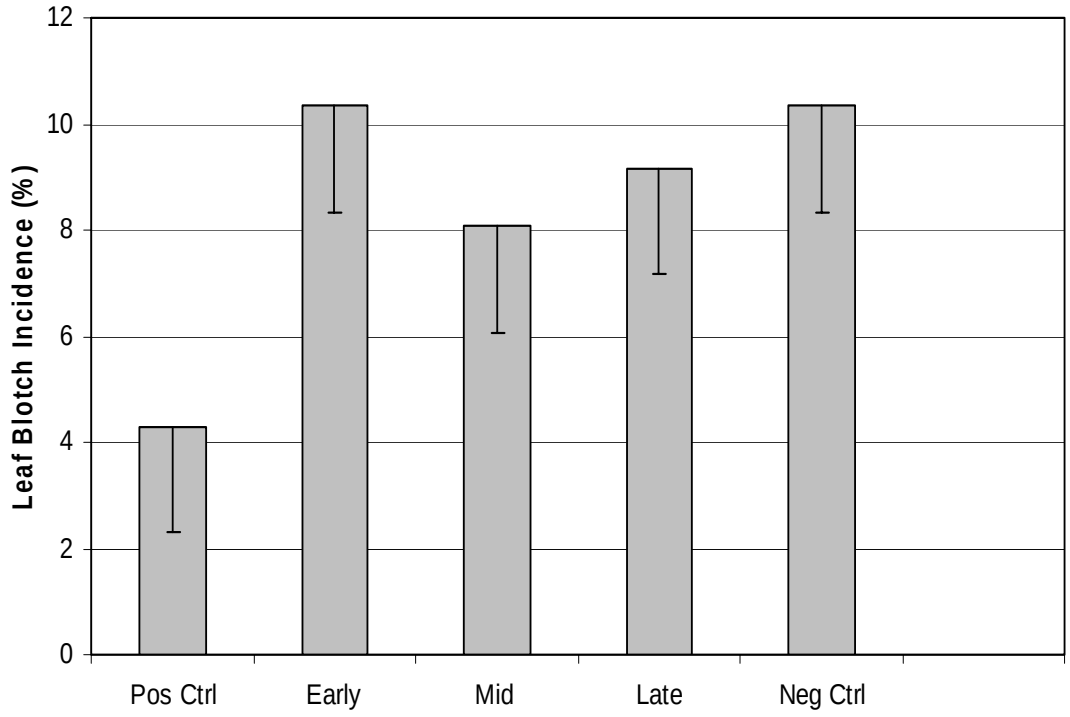


Figure 10.2 Incidence of leaf blotch (var. Festival) on strawberry mother plants from prochloraz applied treatments. Bars are the least significance difference where p=0.05.

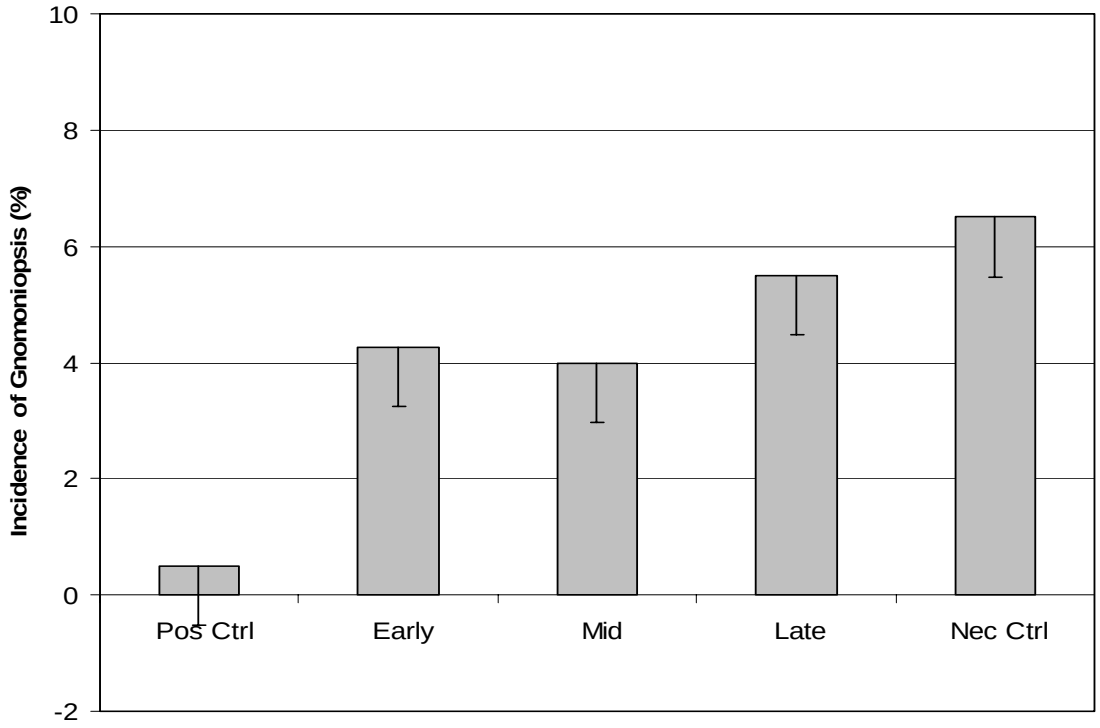


Figure 10.3. Incidence of *Gnomoniopsis* (var. Festival) in petioles sampled from various prochloraz applied treatments. Bars are the least significance difference where p=0.05.

The incidence of leaf blotch on strawberry fruit plants in Queensland and Victoria was low (<2%), and there were no significant differences between treatments (data not shown). There were no symptoms of stem end rot on fruit observed in the trials, and no incidence of *Gnomoniopsis* in plants at the end of the trial.

Prochloraz alternatives trial

All fungicide treatments reduced the incidence of leaf blotch and spot compared with the untreated control, except Cyprodinil + Fludioxonil (Table 10.11). In the mother generation, where the leaf blotch pressure was high, Azoxystrobin + Difenoconazole gave better disease control than the industry standard Prochloraz. Results for disease severity and *Gnomoniopsis* incidence showed similar trends to those for disease incidence (data not shown). There was no significant difference in yields between treatments (data not shown).

Table 10.11. Mean disease incidence recorded on leaves of mother plants (leaf blotch) and runners (small dark spots) per plot for all the treatments at harvest after final fungicide application. Mean values followed by different letters in each column are significantly different, $p \leq 0.05$.

	Mean Disease incidence (%)	
Fungicides treatments	Mother Generation (2011)	Commercial Generation (2012)
Azoxystrobin + Difenoconazole	8.0 a	3.47 a
Pyraclostrobin	16.9 b	6.86 b
Boscalid + Pyraclostrobin	17.0 b	8.83 b
Prochloraz (Positive control)	19.6 bc	3.24 a
Rotation	21.6 c	12.72 c
Trifloxystrobin	21.7 c	12.03 c
Unsprayed (Negative control)	26.0 d	15.34 d
Cyprodinil + Fludioxonil	26.9 d	17.07 d
l.s.d. (0.05)	3.2	1.983

Spray program trial

Fungicide formulations used as stand-alone treatments or in combination with other formulations (e.g. Programs 1 & 2) significantly reduced leaf blotch and spot incidence in runners compared with the untreated control (Table 10.12). Spray program 2 (Azoxystrobin + Difenoconazole as the primary fungicide) was more effective than spray program 1 (Prochloraz as primary fungicide) in reducing disease incidence.

Prochloraz was effective in controlling disease incidence when applied on its own. When combined with Boscalid + Pyraclostrobin in spray program 1, however, disease control reduced significantly.

Table 10.12. Mean disease incidence recorded on leaves of mother plants (leaf blotch) and runners (Small dark spots) per plot for all the treatments at harvest after final fungicide application. . Mean values followed by different letters in each column are significantly different, $p \leq 0.05$.

Fungicide application programmes treatment	**Leaf blotch Incidence (%)**	**Small dark spots Incidence (%)**
Unsprayed *(Neg)*	16.87 e	0.90 c
Prochloraz *(Pos)*	7.06 b	0 a
Prochloraz (P1 control)	6.42 b	0.10 a
Program 1	12.0 d	0.0192 a
Boscalid Pyraclostrobin (P1 control)	12.47 d	0.267 b
Azoxystrobin Difenoconazole (P2 control)	0.87a	0 a
Program 2	0.67 a	0 a
Cyprodinil Fludioxonil (P2 Control)	9.08 c	0.242 b

10.4 Discussion

10.4.1 Effects of environment on leaf spot disease by Gnomoniopsis

A controlled-environment study showed that higher temperatures (16ºC and 26ºC) under high humidity favoured the development and expression of leaf blotch disease, but not the infection and colonisation of strawberries by *Gnomoniopsis fructicola* (causal organism). Recovery of the organism from inoculated plantlets subjected to low temperatures (10ºC) and high humidities suggested that the fungus can infect and colonise the host without producing any visual symptoms. Disease incidence and increased severity due to change in temperature, also indicated that temperature had an effect on either the host or the pathogen or both.

In artificially inoculated plantlets, small dark spots (SDS) started appearing on lateral veins and between veins, and by 6-7 days the spots could be seen on the top of leaves. The spots coalesced and eventually the whole leaf became necrotic. Some of the older leaves in the inoculated plantlets did not express any symptoms but new emerging leaves that were at bud stage during inoculation showed SDS symptoms when fully expanded. Small lesions appeared on petioles at the late stage of infection and the lesions developed quickly along the petiole. Symptoms observed (SDS) in controlled environment experiment were different to symptoms seen in field infections (leaf blotch and spots). Similar observations have been made in previous controlled environment studies with *G. fructicola* / strawberry pathosystems conducted overseas. Later field observations in the runner and fruit industries revealed that young plants were showing SDS symptoms on the under surface of the leaves but older leaves had only blotch symptoms. It is likely that SDS symptoms indicate early infection of the plant, and may provide growers a good indicator of when to treat their plants.

In this study it was not clear if the temperature had an effect on the host or on the organism in causing disease infection and expression. Further research is required to

fully understand the relationship between host, pathogen and environment in this disease.

10.4.2 Control of Gnomoniopsis in the runner industry

Laboratory and field experiments in these studies showed the strong potential for strobilurin fungicides to supplement the use of Prochloraz for control of leaf blotch and *G. fructicola* in strawberry runners. For example, Azoxystrobin + Difenoconazole and Pyraclostrobin showed similar or better efficacy to Prochloraz against leaf blotch and *G. fructicola* in all the field and *in vitro* trials. Furthermore, Azoxystrobin + Difenoconazole was fungicidal against mycelium of *G. fructicola* (rather than fungistatic) in a similar manner to Prochloraz.

In vitro studies have shown that fungicide application technique is critical in determining the efficacy of the chemical. The mode of action of the fungicides is crucial in determining the best application technique. For example a contact fungicide requires more complete coverage of the plant than a fungicide with some systemic activity. For example, the hyphal transfer tests showed that *G. fructicola* can recover and grow even if a very small fraction of the mycelium is left untreated.

Currently, Prochloraz remains the most important tool available for controlling *Gnomoniopsis* in strawberry runners. Field and laboratory trials both showed that Azoxystrobin + Difenoconazole (strobulurin + azole) and Pyraclostrobin (strobulurin) as potential alternatives to Prochloraz (azole). Strobilurins and azole groups have different modes of action against fungi and therefore may provide better control than reliance on a single chemistry alone. Use of combinations of fungicides from different activity groups is the preferred option as this may also reduce the risk of pathogens developing resistance to them. Fungicide program trials showed that growers have at least two chemical groups which they can use in conjunction with other fungicides from other groups in a rotation spray schedule.

Complementary trials are needed in both the strawberry runner and fruit sectors, because management of *G. fructicola* will need a whole-of-industry approach. The results from these trials will assist in planning better control systems for the whole strawberry industry.

11. Technology Transfer (Activity 4)

11.1 Objectives

In conjunction with research, this project conducted a technology transfer program that aimed to achieve three major outcomes:

1) Clear pathways established for the registration and regulatory approval of alternatives to MB.

Target Audience:
Government (e.g. APVMA, OCPPO) and grower agencies (VSICA) responsible for registration, market access, and certification approval of alternatives to MB.

Communication Message:
Scientific research (technical data) shows that alternatives, such as MI, have equal efficacy to MB for soil disinfestation and runner production, and minimise the risk of transporting pathogens and weeds in strawberry transplants sent around Australia.

Desired Response:
Regulatory authorities provide clear pathways and directions to expedite the registration and approval of alternatives to MB.

2) Rapid adoption of MB alternatives by the runner industry following registration and approval.

Target Audience:
Strawberry runner and fruit growers.

Communication Message:
New alternatives are in development that have equal efficacy to MB for soil disinfestation and the production of high-health runners.

Desired Response:
Increased awareness and willingness of growers to trial new alternatives on their properties to prepare them for rapid adoption following registration and approval.

3) Informed decisions on critical-use nominations for MB in the Australian runner industry.

Target Audience:
Industry (TCSRGC, VSICA); government (DEHWA); and UN (MBTOC) agencies responsible for the application and assessment of critical use nominations for MB in Australia.

Communication Message:
Scientific research (technical data) shows that alternatives, such as methyl iodide, have equal efficacy to standard rates of MB for soil disinfestation, but these products need registration and approval before industry can adopt them.

Desired Response:
Informed applications and decisions on critical use nominations, and systematic reductions in MB use by the Australian runner industry.

11.2 Method and outputs

To achieve the above outcomes, the project employed a variety of communication tools ranging from grower days and farm walks to government reports and scientific papers. Over its duration (5 years), this project delivered: 21 grower newsletter articles and manuals, 19 oral presentation days to growers and industry, 17 technical reports to government agencies, 27 scientific conference papers, 1 university thesis, 7 refereed scientific papers and 2 book chapters (see below).

Industry Newsletters:

Gounder, R.K. et al. (2011). Management of leaf blotch in strawberry runners. Flyer distributed to all Victorian strawberry runner growers (29/11/11).

Mattner, S.W. et al. (2011). Recycling carbon waste for soil fumigation. *Nordiko News*

Godwin S. (2009). Strawberry Search for New Fumigant. The Weekly Times, 22 Jul 2009, page 76.

Horner, I.J. (2009). Soil fumigation trials. Strawberry Growers NZ Newsletter, March 2009.

Horner, I.J. (2009). Soil fumigation. Strawberry Growers NZ Newsletter, August 2009.

Mattner, S.W. et al. (2009). Finding alternatives to methyl bromide. HAL Annual Industry Report 08/09, Strawberry, page 3.

Mattner S.W. et al. (2009). Safeguarding the high health status of planting material. HAL Annual Industry Report 08/09, Strawberry, page11.

Wharton, E. et al. (2009). Berry Industry Expo and Farmwalk. VicStrawberries, Issue 33, page 4

Horner, I.J. (2008). Soil fumigation update. SGNZ Newsletter, April 2008.

Horner, I.J. (2008). Soil fumigation trials. Strawberry Growers NZ Newsletter, November 2008.

Mattner, S.W. et al. (2008). Breaking critical use barriers preventing the strawberry industry from phasing out methyl bromide. Wild About strawberries, Issue 16, page 7.

Mattner, S.W. et al. (2008) Methyl bromide (MB) alternatives project update. VicStrawberries, Issue 28, page 2.

Mattner, S.W. et al. (2008) Methyl bromide: Going, going gone! Flowers Victoria Newsletter (December).

Mattner S.W. (2008). Safeguarding the high health status of planting material in the Australian strawberry industry. HAL Annual Industry Report 07/08, Strawberry, page 5.

Wharton, E. et al. (2008). VSGA AGM and farmwalk. VicStrawberries, Issue 30, page 1.
Horner, I.J. (2007). Soil fumigation update. SGNZ Newsletter, November 2007.
Mattner S.W. et al. (2007) Status on critical-use exemptions for methyl bromide in the Australian strawberry industry. Strawberries Australia 2006/07 Annual Report.
Mattner S.W. et al. (2007) !!Warning: Planting too early into fumigated soils can kill your strawberry crops!!. Flyer distributed to all fruit growers purchasing TCSRGC strawberry runners.
Porter et al. (2007). Strawberry industry award – 2007 Best of the Best Stratospheric Ozone Protection Awards. Wild About Strawberries, Issue 14, pg 5.
Thomson C. (2007) VSGA Farmwalk October 2007. VicStrawberries Issue 27, page 4.

Grower Manual:

Horner, I.J. et al. (2008). Beyond Methyl Bromide Soil Fumigation: A Guide for New Zealand Strawberry Growers. Manual produced for Strawberry Growers NZ Inc. 35pp.

Grower Presentations:

Strawberry runner grower day. Two oral presentations: 'Update on alternatives to MB' (SW Mattner) and 'Control of *Gnomoniopsis* diseases in the runner industry' (RK Gounder). Toolangi, 22 March 2012.

Update critical use exemptions in the runner industry. VSICA AGM, Toolangi, 29 November, 2011.

Environmental and bystander safety of the soil fumigant methyl iodide. Oral presentation to Australian Pesticides and Veterinary Medicines Authority, Office of Chemical Safety and Environmental Health, Department of Sustainability, Environment, Water, Population and Communities, invited runner growers, and Arysta LifeSciences. APVMA, Canberra, 24 October 2011.

Detection and control of leaf blotch and stem end rot of strawberries caused by *Gnomoniopsis fructicola*. Oral presentation, La Trobe University Bundoora, Vic, 12 May 2011.

Field walk and oral presentation at *Gnomoniopsis* trials. Attended by 35 runner and fruit growers from Vic, WA, and Qld. Millgrove, Victoria, 18 December 2010

Managing weeds in strawberry fruit production with MB alternatives. Oral presentation to 30 strawberry fruit growers. Landmark, Wandin, Victoria, 17 November 2010.

MB critical-use meeting. Oral presentation and meeting with representatives from Department of Environment and Heritage, chemical companies, fumigators, strawberry runner growers, and runner certification authorities. Knoxfield, Victoria, 27 October 2010.

Disease management in horticulture with MB alternatives. Oral presentation to 15 horticultural growers. Harcourt, Victoria, 30 July 2010.

Three oral presentations, 'MB research update', Scott Mattner; 'Maintaining biosecurity in the strawberry runner industry – *Gnomoniopsis* as a model', Rajendra

Gounder; ‘Soil-less systems as an alternative to MB for foundation stock production’, Linda House. Victorian Strawberry Industry Certification Authority AGM, Toolangi DPI, November 2009.

One field demonstration ‘MB alternatives trial walk’, Rajendra Gounder and three oral presentations ‘Alternative fumigants for soil disinfestation in the strawberry industry’, Scott Mattner; ‘Strawberry production and soil disinfestation in the Spanish strawberry industry’, Belen Guijarro; and ‘The importance of soil health for strawberry production’, Scott Mattner. Third National Berry Industry Expo, Lilydale and Millsgrove, Vic, July 2009.

Field day and strawberry fruit and runner grower workshop. The workshop included two oral presentations, ‘Sustainable Soil Management Without Methyl Bromide (NZ)’, Ian Horner, and ‘Soil disinfestation for strawberry production (Australia)’, Scott Mattner; one laboratory demonstration of strawberry pathogens and measuring fumigant residues in soil (Ian Horner); field demonstration of drip fumigation; a tour of commercial fumigation facilities; and two field walks of soil disinfestation trials (Ian Horner and Scott Mattner). Strawberry Growers New Zealand Inc Annual Conference, Havelock North, NZ, June 2009.

Two oral presentations. ‘Alternatives to MB for flower production’, Scott Mattner; ‘MB phase-out: the international scene’, Ian Porter. Flowers Victoria AGM, Bayswater, Vic, December 2008.

Field day and strawberry runner grower workshop. Four oral presentations, ‘MB Critical Use Nominations’, Peter Merriman; ‘MB phase-out: the international scene’, Ian Porter; ‘MI registration process’, Ian Crook; ‘MI research update for strawberry runners’, Scott Mattner and group discussion. Toolangi Strawberry Runner Growers Co-operative, DPI Toolangi, Vic, October 2008.

Oral presentation, ‘Alternatives to methyl bromide for strawberry fruit production’, Ross Mann. Victorian Strawberry Growers Association Farm Walk, Main Ridge, Vic, October 2008.

Two oral Presentations, ‘Update on MI research: strawberry runners (Scott Mattner) and strawberry fruit (Ross Mann)’. Victorian Strawberry Industry Development Committee, Lilydale, Vic, August 2008.

Oral Presentation, ‘Sustainable Soil Management Without Methyl Bromide’, Ian Horner. Strawberry Growers NZ Inc Annual Conference, Auckland, NZ, June 2008.

Industry presentation, ‘Soil fumigants and soil health’. Victorian Strawberry Industry Development Committee and the Victorian strawberry IDO, Knoxfield, Vic, April 2008.

Oral presentation, ‘Methyl iodide and drip fumigants for soil disinfestation in the strawberry industry’, Scott Mattner. Victorian Strawberry Growers Association Farm Walk, Gruyere, Vic, October 2007.

Oral presentation and information booth, ‘Alternative fumigants and soil health’, Scott Mattner. Second National Berry Industry Expo, Lilydale, Vic, August 2007.

Industry Reports:

Mattner et al. (2012). Iodane 500. Bystander safety trial, Toolangi Victoria. Report for APVMA.

Rose et al. (2012). Methyl iodide bystander trial – method validation report. Report for APVMA.
Allison et al., (2011). Methyl iodide fluxes from chamber studies at Toolangi, Victoria, Australia. Report for Project BS07014, August 2011.
Mattner et al., (2011). Efficacy of recaptured methyl bromide from quarantine applications for soil disinfestation in the strawberry runner industry. Summary report for project BS07014, August 2011.
Reiss et al. (2011). Analysis of air monitoring data at iodomethane and chloropicrin field trial in Toolangi, Victoria in Australia. Report for APVMA.
Mattner et al. (2010). Trial protocol: Methyl iodide bystander safety trial. Report to APVMA.
Horner, I.J. (2009). Strawberry culture beyond methyl bromide. Report prepared for Strawberry Growers New Zealand Inc. and MAF Sustainable Farming Fund, SFF Project 07/119. Plant & Food Client Report Number 25381, Plant & Food Contract No. 22612. October 2009.
Horner, I.J. (2009). Strawberry culture beyond methyl bromide. Report prepared for Strawberry Growers New Zealand Inc. and MAF Sustainable Farming Fund, SFF Project 07/119. Plant & Food Client Report Number 25380, Plant & Food Contract No. 22612. June 2009.
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Horner, I.J. (2008). Strawberry culture beyond methyl bromide. SFF Report Feb. 2008. HortResearch Client Report No. 25376. HortResearch Contract No. 22612
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Horner, I.J. (2007). Strawberry culture beyond methyl bromide. SFF Progress Report Project 07/119, October 2007. HortResearch Client Report. HortResearch Contract No. 22612.

Scientific Conference Papers:

Gounder R.K. et al. (2012) Control of leaf blotch caused by *Gnomoniopsis fructicola* in strawberry runners. Seventh International Strawberry Symposium, Beijing, China.

Mattner et al. (2012) Alternatives to methyl bromide for soil disinfestation in the Australian strawberry nursery industry. Seventh International Strawberry Symposium, Beijing, China.

Gounder R.K. et al. (2010). Incidence and control of *Gnomoniopsis* fructicola in strawberry transplants. Poster. AgriBio Conference, La Trobe University, 29 November, 2010.

Gounder R.K. et al. (2010). Detection and control of leaf blotch and stem end rot of strawberry caused by *Gnomoniopsis* fructicola. Australasian Plant Pathology Conference.

Mann, R.C. et al. (2009) Novel biological methods for crop protection against soilborne pathogens derived from endophytic fungi. Proceedings of the 5th Australasian Soilborne Diseases Symposium. pp 99-100. Thredbo, NSW, 5-7 February 2009.

Mann, R.C., Horner, I.J., et al. (2009) Improving the efficacy and safety of soil disinfestation in horticulture with drip fumigation systems. Proceedings of the 5th Australasian Soilborne Diseases Symposium. pp 53-55.

Mattner, S.W. et al. (2009) Maintaining biosecurity and market access in the Australian strawberry industry following methyl bromide phase-out. In Proceedings of the VII International Symposium on Chemical and non-Chemical Soil and Substrate Disinfestation. pp 25. Katholieke Univeriteit Leuven, Belgium, 13-18 September 2009.

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Porter, I.J. et al. (2009). Increasing global regulations on fumigants stimulates new era for plant protection and biosecurity. In Australasian Plant Pathology Society Conference 2009. pp 121. Newcastle, NSW, 29 September – 1 October 2009.

Porter, I.J. et al. (2009) Maintaining biosecurity and market access in the Australian strawberry industry following methyl bromide phase out. Annual International Methyl Bromide Alternatives and Outreach Conference, p 4:1. November 2009, San Diego.

Porter, I.J. et al. (2009) Progress in global phase-out of methyl bromide and the relative effectiveness of soil disinfestation products to replace fumigants for preplant soil uses. In Proceedings of the VII International Symposium on Chemical and non-Chemical Soil and Substrate Disinfestation. pp 19. Katholieke Univeriteit Leuven, Belgium, 13-18 September 2009.

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11.3 Evaluation

The success of the communication program in achieving its desired responses and outcomes can be evaluated by the following factors:

1) A pathway for the possible approval of new soil fumigants, such as methyl iodide, and disinfestation practices in the runner industry was outlined.

This project communicated technical data on alternative fumigants (particularly methyl iodide) to: (1) the Office of the Chief Plant Protection Officer to support decision-making on the interstate market access of runners grown in soils treated with alternatives, (2) strawberry runner authorities to allow decisions on the approval and inclusion of alternative fumigants in Australian certification schemes, and (3) the APVMA to support the possible registration of alternatives in the in the runner industry. This communication model is currently being followed by prospective registrants of new alternative fumigants (e.g. ethanedinitrile, dimethyl disulphide) because it is acknowledged as the fastest way of getting adoption in the runner industry.

2) Growers are aware and trialling methyl iodide, and adopting other alternatives where possible.

At a recent workshop in the runner industry conducted in 2012, 100% of growers questioned were aware that methyl iodide offered an effective alternative to MB for soil disinfestation, but were concerned about lack of information on cost. To date, 80% of Australian runner growers have trialled methyl iodide or other alternatives to MB on their farms – an increase of 60% since the commencement of this project.

In the strawberry fruit industry, two new alternative fumigants (Telone C60® and PicPlus®) were registered during the course of this project. These applications were supported by data from this and previous projects. These two alternatives have now become the two most widely adopted fumigants in the fruit industry. In addition, the application technique of drip fumigation investigated in this project was recently registered for some new alternatives (InLine®) in the fruit industry. Adoption of this practice in the industry is still small, but seems likely to increase in areas suited to its application.

3) Informed applications and decisions have been made on critical-use nominations for MB in the Australian runner industry.

During the course of this project, MB use in the Australian runner industry has fallen by 17% (from 35.75 to 29.76 tonnes p.a.). This reduction resulted from the identification of regions and strawberry varieties that could tolerate the use of existing alternatives, such as 1,3-D:Pic (Telone®) mixtures. It also resulted from the use of soil-less production systems for producing runners in the early stages of the multiplication system. The runner industry have identified methyl iodide as their best opportunity to phase out MB provided it becomes registered, and have outlined a phase-in adoption plan in their annual CUE applications. This project has communicated technical data to the runner industry, the Department of Sustainability, Environment, Water, Population and Communities (agency responsible for implementing the Montreal Protocol) and the UN Methyl Bromide Technical Options Committee to ensure that all decisions on CUEs in Australian runners were based on science.

12. Recommendations

The research reported here demonstrated that methyl iodide can provide the same level of biosecurity and certification standards for strawberry runner production as methyl bromide and would be an acceptable alternative. Data was submitted to APVMA to expedite approvals for registration, market access and use in certification schemes. However, since completing this work, Arysta has notified APVMA in June 2012 that it is withdrawing the application for registration of methyl iodide due to the current high cost of iodine worldwide. Therefore, there is an urgent need to find another alternative to methyl bromide, otherwise phase out will not be possible without compromising biosecurity and market access standards in the runner industry.

The re-use of methyl bromide captured from quarantine and preshipment use was examined and showed potential. Similarly, the use of impermeable barrier films has potential to improve the efficacy of some products. With further research, the alternative ethanedinitrile, identified in a previous project (BS01004), may also provide industry with an opportunity to phase-out methyl bromide. Further research should also be directed towards developing integrated disinfestation systems that combine existing fumigant, fungicide, nematicide, herbicide and biofumigant chemistries for control of soil-borne pests in the runner industry. Further research, however, is necessary to validate that these can provide the biosecurity levels required for runner production and interstate movement of planting material. Development and registration of any products will require a coordinated effort between private companies, federal agencies, Strawberries Australia, TSRGC and the key research agencies.

In the face of this, industry and government need to consider:

(1) ongoing applications for critical use exemptions for methyl bromide (note: this would require ongoing research programs to find alternatives) while MB alternatives are being developed, or

(2) accepting the risks of reduced biosecurity levels in runners with current alternatives and determining legal responsibility for this risk with federal and state governments, certification agencies, and runner and fruit industries.

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