Efficacy and suitability of liquid ethyl formate for insect pest management

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By

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Declaration

I declare that this thesis is my own account of my research and has not been submitted for a degree at any tertiary education institution.

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Attribution Statements

The content in this thesis was developed by the Candidate with advice from their supervisory panel.

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Abstract

The Australian agricultural industry is critical to the nation's economy. However, it is under significant threat from insect pests that damage crops both pre- and post-harvest. As a result, quarantine/biosecurity treatments in the form of fumigations form the vanguard of Australia's defence against insect pests, both established and threatening to establish. The brown marmorated stink bug, an invasive pest yet to establish in Australia stands to cause enormous financial damage to multiple crops, an example being this thesis' estimated cost over $300 million worth of damage to Australian wine grape production alone should poor biosecurity allow its establishment. Currently employed biosecurity treatments such as heat treatment, methyl bromide and sulfuryl fluoride are flawed when it comes to this pest. This has prompted evaluation of the organic and food-grade compound ethyl formate. Ethyl formate trials found that Probit 9 could be achieved in low temperatures against tolerant diapausing brown marmorated stink bug at 23.51 mg/L. It was then important to evaluate the potential of ethyl formate to protect one of Australia's most valuable agricultural exports, grain. Currently the export grains value chain relies on phosphine to meet federally mandate nil-tolerance for live insects, but this future of this fumigant is threatened by resistance development. The simulated grain silo bioassay found that grain sorption of the fumigant did not prevent ethyl formate from effectively controlling adult stored grain insect pests. Finally, it was important to evaluate the potential inter-reaction between ethyl formate and representative materials / products encountered in biosecurity treatments. Results from this evaluation found no deleterious effects on the integrity and function of various plastics and metals. In conclusion, ethyl formate was shown to be a safe, reliable and effective fumigant with a range of applications, and needs to be considered as potential mainstay biosecurity treatment for both import and exports.

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Chapter 1: General introduction and literature review

1.1. General introduction

The Australian agricultural industry contributes $81 billion to the national economy (Cameron, 2022). This continues to grow each year and is thanks to the absence of many invasive pests that have ravaged agriculture in other parts of the world (Inspector-General of Biosecurity, 2018). The Australian Government describes an invasive species as "a species occurring, as a result of human activities, beyond its accepted normal distribution and which threatens valued environmental, agricultural or other social resources by the damage it causes" (Department of the Environment and Energy, 2017). The last 30 years have brought about an aggressive rise in the detection of exotic species in territories well outside their natural geographic range (Blackburn et al., 2011). The cause of this is thought to be the ever-increasing global trade network, which has been identified as a carrier for countless organisms, providing a wide-reaching pathway to territories that have been historically unreachable (Pimental, 2011). An example of the effects of poor biosecurity in Australia have been the impacts of the red imported fire ant, which has required government to implement an eradication program costing in excess of $400 million (Inspector-General of Biosecurity, 2018). In order to mitigate the risk of threatening to establish exotic insect pests, and those already established, strict biosecurity measures are in place, of which fumigation treatments are the most widely implemented (Australian Chief Plant Protection Office, 2020).

Fumigation is defined as "The action of releasing a toxic chemical in the gaseous state to control a targeted pest" (Federal Grain Inspection Service, 2006). A fumigant is defined as "a toxic, gas phase, chemical agent that is lethal to pests, insects and/or micro-organisms (Hawley, 1987). There are a number of aspects to consider when deploying a chemical agent for fumigation (Derrick et al., 1990):

- The chemical may not only react with living organisms, but with other materials in the fumigation environment;

- The solubility of the chemical along with the gas distribution within the target environment affect the penetration and adsorption of the fumigant;
- The chemical's mode of action and concentration x time product must be suited to the targeted species / life stage;
- The chemical in all likelihood possesses some degree of toxicity towards humans, and will leave a residue following the treatment on any exposed commodity or material.

The key requirements for an effective fumigant are that it controls all life stages of the target pest, remains inert with exposed materials, and there is complete degradation of the compound following aeration of the fumigation chamber (Derrick et al., 1990). A thorough evaluation of the proposed fumigant, together with consideration for its method of application, allows for a more informed decision when selecting the most appropriate chemical for a fumigation treatment.

Through these key attributes of fumigant viability, alternative low molecular weight compounds are currently being evaluated for use in fumigation treatments. These volatile compounds can produce significant vapour phase toxicity and possess a range of potential applications in integrated pest and disease management (Scharf et al., 2006; Nguyen et al., 2014).

A vast array of chemical compounds are highly toxic to insect pests, but most of them are not suitable for application as a fumigant due to their negative environmental, occupational health, and residual effects (Banks, 1990). In recent years, proven fumigants have seen reduced usage; for example methyl bromide has been banned in many situations due to its ozone-depleting properties (United Nations Environment Programme, 2006). Phosphine, the only widely available fumigant accepted by world trade, has fallen out of favour due to insect

resistance (as a result of poor fumigation practices), and the length of time required to reach end-point mortality (Xin et al., 2008). This has posed a unique challenge to fumigation chemists throughout the world, leading them to investigate alternative fumigants. Ethyl formate is one such fumigant and has been identified as showing great potential (Ren and Mahon, 2006).

1.2. Principles of fumigation

The boiling point of chemical compounds has a directly proportional relationship with its molecular weight, excluding methyl bromide (Table 1-1). This relationship demonstrates that low molecular weight compounds such as ethyl formate (74.08 g/mol) will evaporate rapidly under practical fumigation conditions. In the case of high molecular weight compounds such as carbon tetrachloride (153.82 g/mol) evaporation is slow; and as such, rapid vaporisation has to be brought about via alternative means that will be discussed below.

Table 1-1. Molecular weight and boiling point of common fumigants according to Bond and Monro (1984).

Name (formula)	Molecular weight (g/mol)	Boiling point °C (at 760 mm pressure)
Dichlorvos ($C_4H_7Cl_2O_4P$)	221.00	120.00
Ethylene dibromide (CH_2BrCH_2Br)	197.88	131.00
Chloropicrin (CCl_3NO_2)	164.39	112.00
Carbon tetrachloride (CCl_4)	153.84	77.00
Sulphuryl fluoride (SO_2F_2)	102.60	-55.20
Ethylene dichloride ($C_2H_4Cl_2$)	98.97	83.00
Methyl bromide (CH_3Br)	94.95	3.60
Ethyl formate ($C_3H_6O_2$)	74.05	54.00
Methyl formate ($C_2H_4O_2$)	60.03	31.00
Ethylene oxide (C_2H_4O)	44.05	10.70
Phosphine (PH_3)	34.04	-87.40
Hydrogen Cyanide (HCN)	27.03	26.00

The maximum weight of a chemical that can exist as a gas in a given space is dependent upon its molecular weight (Bond and Monro, 1984). This notion is supported by Avogadro's law, which states that two gasses of equal volume, at the same temperature and pressure, contain the same number of molecules (Vitz et al., 2019). In a practical setting, it is therefore ineffective trying to volatilise more fumigant in an empty chamber than can exist as a vapour. Sorption of the fumigant by the material treated in a given space will permit greater amounts to be volatilised over time.

Diffusion in a fumigation context refers to the movement of individual gas molecules over distances less than 1 mm (Banks, 1990). According to Graham's Law of Diffusion, the velocity of diffusion of a gas is inversely proportional to the square root of its density (Piper and Worth, 1980). The density of a gas is also proportional to its molecular weight. As a result, a high-density gas like ethylene dibromide (197.88 g/mol) will take longer to diffuse through an open space than a comparatively lighter gas in ethylene oxide (44.05 g/mol). However, the principles of diffusion governed by Graham's Law are basic, and do not take into account various other factors encountered in a practical controlled atmosphere such as sorption and temperature, which are discussed below.

Most compounds used for fumigation are heavier than air, and as a result, when introduced to a still atmosphere, will sink and form a layer below air (Bond and Monro, 1984). This phenomenon has been observed in practice, whereby a fumigation treatment of insects in a ship hold delivered high mortality in the bottom section but was largely ineffective in the upper sections (Monro et al., 1952). As a result, fumigators have sought to employ various forced flow and air circulation methods, along with the inclusion of a lighter than-air carrier gas with the fumigant (Bond and Monro, 1984; Krishna et al., 2005). Once proper mixing between the air and fumigant has occurred, heavier than-air compounds sink to form a layer

below the air at such a slow rate that its impact is negligible over the typical fumigation period.

Methods of air circulation such as fans and blowers can be highly effective in accelerating both the distribution and penetration of the fumigant (Bond and Monro, 1984). Air circulation methods can be utilised either within the fumigation chamber or via vents from outside. The efficiency of the entire fumigation treatment is greatly improved by the increasing of the rate of volatilisation of high boiling point liquid fumigants from evaporating pans and preventing the stratification of heavy compounds once evaporated (Bond and Monro, 1984). Rapid stirring of gas from air circulation devices has also been documented to assist in the protection of vulnerable materials (food product) exposed to the fumigant by ensuring that they are not directly exposed to excessively high concentrations (Lubatti and Bunday, 1958).

Sorption can be defined as "a phenomenon of fixation or capture of a gas or a vapour (sorbate) by a substance in condensed state (solid or liquid) called sorbent" (Hauer, 2007). This process takes away some of the fumigant compounds molecules from the free space, preventing them from diffusing freely throughout the chamber, and limiting their ability to penetrate further into the commodity (Bond and Monro, 1984). During a fumigation treatment, air and gas molecules collide, causing a deceleration of diffusion through the commodity, resulting in sorption being a gradual process (Bond and Monro, 1984). Thus, the decrease of fumigant concentration in the free space of the chamber is progressive.

Sorption plays a key role in determining fumigant dosage for the treatment. This is because the fumigant dosage must be high enough to cover the total amount of sorption, with enough fumigant remaining to achieve the desired mortality level for the target organism (Bond and Monro, 1984). Sorption can take place as both a physical and a chemical process.

Physical sorption or physisorption, refers to “the process of mechanically locking up molecules in other solid material” (Welker, 2012). Specifically, physisorption refers to the processes of adsorption and absorption, which involve weak and reversible van der Waal’s forces (Pashin, 2008). Adsorption occurs when gas molecules stay attached to the surface of a material. As some fumigated commodities such as grain or charcoal are highly porous, adsorption increased adsorption can take place due to the increased surface area (Banks (Banks, 1990). Absorption occurs when the gas becomes a solid or liquid and is held by capillary forces of the commodity that govern the properties of solutions. For example, a gas may be absorbed in the aqueous phase of grain or in the lipid phase of nuts, cheese or other fatty foods (Berck, 1964). Physical sorption is also attached to the concept that a greater amount of sorption occurs with high boiling point compounds in comparison to more volatile ones (Bond and Monro, 1984). Physical sorption has an inverse relationship with temperature, meaning that as the temperature rises, the rate of sorption decreases and vice versa (Bond and Monro, 1984). This is a key reason why fumigant dosages generally have to be progressively increased as the temperature of the fumigation is lowered.

Sorption may also be influenced by the moisture content of the commodity being fumigated. Lindgren and Vincent (1962) documented a positive relationship between the sorption of the fumigant methyl bromide, and moisture content within the fumigation chamber.

However, known laws and generalisations are unable to predict the specific reaction between gases and commodities. As a result, the fumigant must always be tested with every material it will encounter for accurate fumigation procedures to be decided.

Chemical sorption or chemisorption “involves the transfer, exchange, or sharing of electrons between adsorbates and adsorbents (atoms or molecules), and the adsorption of adsorbates on adsorbents is due to the formation of chemical bonds between them” (Deng et al., 2019).

Chemisorption is a stronger form of bonding compared to physical sorption, resulting in a chemical reaction between the gas and the material, and is irreversible under ordinary circumstances (Berck, 1964).

Examples of chemisorption are the reaction between methyl bromide and wheat; where the compound is broken down in wheat to form several non-toxic derivatives (Winteringham et al., 1955); and hydrogen cyanide, which can combine with sugars in dried fruit to form laevulose cyanohydrin (Page and Lubatti, 1948). Another example is the reaction between hydrogen cyanide (HCN) and the reducing sugars in dried fruits, producing a cyanohydrin (Page and Blacklith, 1956); or the formation of inorganic bromide compounds following a fumigation treatment of foodstuffs with methyl bromide (Monro, 1941).

It has been suggested that the intensity of reaction and temperature are positively related, in that more of a compound is volatised at a higher temperature, resulting in greater residue. This notion is supported by Dumas (1973), who found equitably less bromide residue in fruits as the ambient temperature in fumigation chamber was lowered from 25 to 4 °C. Research conducted by Lindgren et al. (1962) also found higher levels of methyl bromide residue in fumigated wheat as the temperature was increased from 10 to 32 °C.

Following a fumigation treatment, the chamber is ventilated, removing any remaining gas, and allowing the fumigant to gradually be removed from the treated commodity (Bond and Monro, 1984). This process is referred to as desorption and contrasts the process of physical sorption. The length of time necessary for total desorption is heavily dependent upon the fumigant and material treated. Because of the inverse relationship to temperature, complete decay of the fumigant typically occurs at a slower rate in a cooler environment (Bond and Monro, 1984). Therefore, it is suggested that heating of the fumigation chamber and

commodity will promote an accelerated breakdown of the compound and its residue (Bond and Monro, 1984).

Humidity is also a critical factor effecting the fumigant desorption. A fumigation treatment of wheat using ethylene dibromide at high humidity (75-85% relative humidity [RH]), found the fumigant desorbed 80 percent more than at low humidity (0% RH) (Dumas and Bond, 1979). Due to humidity changing considerably with temperature, the combination of both these factors can significantly influence the rate of desorption. The venting of desorbing gas can be accelerated by the use of industrial fans which circulate outside air throughout the chamber and commodity. However, the chamber / silo can be opened to allow for natural ventilation, or the commodity may be completely removed from the fumigation chamber (Bond and Monro, 1984). Due to the irreversible process of chemical sorption, it is possible for small quantities of the fumigant to not desorb from the commodity.

According to the current knowledge on fumigant modes of action, the compound enters the insect via the respiratory system. The primary orifice for the fumigant to enter through in most life stages (larvae, pupae, nymph, adult) are the spiracles, which are located on the lateral surfaces of the insect body, and under muscular control. For fumigants to penetrate egg stage insects, it must first diffuse through the shell into the pre-emergent insect (Bond and Monro, 1984). Therefore, with respiration being the key target for most fumigants, anything that increases an insect's respiration rate will make the fumigant more effective (Lu et al., 2009).

Temperature is the most significant environmental factor influencing the effect of a fumigant. Within the typical temperature range for fumigation between 10 to 35 °C, there is an inverse relationship between the required lethal concentration of fumigant and temperature (Bond and Monro, 1984). From a physiological perspective, this is due to the insects' respiration

increasing in accordance with the increased ambient temperature (Sun, 1946). It has also been documented that physical sorption of the fumigant is inversely proportional to the ambient temperature, meaning that the insect is exposed to a higher amount of the chemical at higher temperatures (Bond and Monro, 1984). Hence, within the aforementioned temperature range, the efficacy of fumigation treatments increase as the temperature rises. Below 10 °C, sorption effects become more prominent whilst the insect's rate of respiration decreases. For example, methyl bromide with a boiling point of 3.56 °C; will take longer to volatilise, but the toxicity is significantly decreased, thus requiring a higher dosage of the fumigant (Bond and Monro, 1984).

Fumigation outcomes can be affected by not only the ambient temperature during the treatment, but also by the temperatures at which the insects are exposed to prior to fumigation. Insects in a cool environment have reduced metabolic rates. If the fumigation is immediately conducted at a higher ambient temperature, the insect's physiological activity may still be significantly reduced, resulting in their uptake of the chemical being limited (Pradhan and Govindan, 1953). This is especially important for insects such as the brown marmorated stink bug (BMSB) that enter a state of dormancy during the winter months known as diapause (Hoebeke and Carter, 2003). Dormant insects can display a level of tolerance towards the fumigant (i.e. methyl bromide or phosphine) not seen in non-diapausing insects (Bell, 1977). It is also well documented that there is a positive relationship between insect respiration and temperature, thereby making fumigations more effective at higher temperatures (Neven, 2000).

Fumigation chambers are designed to allow fumigation to be conducted in an efficient, safe and economical manner. Fumigation chambers can vary in shape and size depending on the specific requirement; however, the basic elements for construction are (United Kingdom Ministry of Agriculture, Fisheries and Food, 1973):

- gas-tight construction;
- an efficient system for fumigant application;
- an efficient system for aeration following treatment; well positioned for convenient commodity handling; and
- improved occupational health and safety for operators.

The key statistical measures of a fumigation efficacy study are the mortality percentage and the level of confidence, which refers to the degree of trust in the level of efficacy (Ormsby, 2018). Regarding a bioassay of the BMSB, the treatment should achieve a mortality percentage greater than 99, with a level of confidence of 95% or above (The Food and Agriculture Organization of the United Nations, 2015). Probit 9 mortality of an insect is another key outcome of a fumigation and has been previously regarded as the gold standard for assessing treatment efficacy (International Plant Protection Convention, 2015). Probit 9 is the equivalent of 99.9968% mortality for phytosanitary treatments, and represents one surviving insect out of over 93,000 (International Plant Protection Convention, 2015)

1.3. Ethyl formate

Ethyl formate (EF), also referred to as ethyl methanoate, formic acid ethyl ester, formic ether, and ethyl formic ester; is a low-molecular-weight volatile compound produced by many fruits and vegetables and has an important role as a flavour and aroma component (National Centre for Biotechnology Information, 2021).

In the last decade, ethyl formate has been successfully deployed as a fumigation treatment for invasive pests in cereals and a variety of fruits such as bananas (Krishna et al., 2005), grapes and citrus (Ryan and De Lima, 2014). The rise of ethyl formate as a fumigant is largely a result of its fast action, strong penetration and degradation to biogenic substances ethanol and formic acid (Muthu et al., 1984; Ren and Desmarchelier, 2002). The threshold limit value

(TLV) for ethyl formate is 100 parts per million (ppm), compared to 3ppm and 0.3ppm for methyl bromide and phosphine respectively. As such, EF is 33 and 333 times less toxic respectively than these primary fumigant competitors (Agarwal et al., 2015). Due to the comparatively low toxicity of ethyl formate, it can be considered as a significantly safer fumigant from both an occupational safety and environmental standpoint.

In Australia, ethyl formate is registered for use as a dried fruit fumigant (Hilton and Banks, 1997; Agarwal et al., 2015); however, the chemical properties of ethyl formate outlined below make it an ideal fumigant for grain and other durable commodities. Research conducted by Xin et al. (2008) assessed the toxicity of gas phase EF with respect to adults of three common durable commodity pests (*Sitophilus oryzae* (Linnaeus), *Rhyzopertha dominica* (Fabricius) and *Tribolium castaneum* (Herbst)) in an empty glass desiccator. Endpoint mortality was achieved within 24 hours of exposure. The results of the study were confounded by the absence of media within the desiccator, negating the ability to determine sorption effects and effect on pre-emergent life stages. Another study conducted within a glass desiccator, examined the sorption effects of grain on the fumigation treatment of the above three pests, reporting a positive relationship between the quantity of grain and the required dosage of fumigant (Damcevski and Annis, 2006).

1.4. Properties and safety considerations of ethyl formate

The U.S. National Library of medicine (National Center for Biotechnology Information, 2021) lists the properties of many chemicals including ethyl formate.

Table 1-2. Summary of the chemical properties for the compound ethyl formate

Chemical properties of ethyl formate
CAS Registry Number: 109-94-4
Molecular Formula: $C_3H_6O_2$
Molecular Weight: 74.08
Melting Point: –80.5 °C
Boiling Point: 54.3 °C
Water Solubility: 118 g/L at 25 °C
Specific Gravity (Water=1): 0.924 at 25 °C
Vapour density (Air=1): 2.55
Vapour pressure: 192 mmHg (25.5 kpa) at 20°C, 300 mmHg (40 kpa) at 30 °C
LEL-UEL: 2.8(3.1-3.6)-16 (15.0-15.7)%
Flammability limit: 84 mg/L (practical data); Max. 12.5 % v/v of EF vapour in CO_2
Flash point: -19.44°C
Conversion factor: 1 ppm = 3.02 mg/m^3 at 25 °C and 1 mg/m^3 = 0.331 ppm at 25 °C
Solubility in other solvents: Ethanol, Ether, Benzene, Acetone.

The U.S. National Library of medicine lists the following health guidelines and limits in place for ethyl formate (National Center for Biotechnology Information, 2021):

- Standard for the Uniform Scheduling of Medicines and Poisons: S6 when ethyl formate is packed and labelled for use as a fumigant
- Australian Pesticides and Veterinary Medicines Authority: The maximum residue limit (MRL) for ethyl formate in the dried fruit industry is 1 mg/kg (ppm)

  Office of Chemical Safety and Environmental Health Acute reference dose - not established.

  No-observed-adverse-effect level - no safety concerns at current levels of intake

  Acceptable daily intake (ADI) - Inchem concluded that ethyl formate could be included in a group ADI for formic acid (0-3 mg/kg body weight)
- Occupational exposures

The National Institute for Occupational Safety and Health: Threshold limit value (TLV) & time weighted average (TWA) – 303 mg/m^3 (8-hr working day, 40-hr week)

- Irritant to mucous membranes, eye, nose, skin

The National Center for Biotechnology Information (2021a) also found ethyl formate has the following effects on humans:

- Minor eye irritation in human subjects and strong persistent nasal irritation were noted on exposure to 330 ppm in the air. The irritating effects on the eyes and respiratory tract are presumably due to rapid hydrolysis of the ester on contact with water, with the formation of alcohol and formic acid. In persons with impaired pulmonary function, especially those with obstructive airway diseases, the breathing of EF might cause exacerbation of symptoms due to its irritant properties. Persons with pre-existing skin disorders may be more susceptible to the effects of this agent. Although EF is not known as a liver toxin in humans, the importance of this organ in the biotransformation and detoxification of foreign substances should be considered before exposing persons with impaired liver function. Although EF is not known as a kidney toxin in humans, the importance of this organ in the elimination of toxic substances justifies special consideration in those with possible impairment of renal function.

Toxicity, metabolism, and pathway studies were also reported by The National Centre for Biotechnology Information (2021), who determined the lethal dose (LD_{50}, acute) was 1850 mg/kg in rats with oral intake and 2000 ml/kg in rabbits with dermal exposure.
EF is absorbed through the lungs, from the gastrointestinal tract, and to a small extent through the skin. This ester is hydrolysed into ethyl alcohol and formic acid with subsequent

metabolism via esterase enzymes, primarily to CO_2 in the case of ethanol, while formic acid is reduced to biologically active methyl, or excreted as the free acid (Haritos et al., 2003). Ethyl formate is used in flavourings for lemonades and essences, in artificial rum and arrac, and as a fungicide and larvicide for tobacco, cereals, dried fruits, and other crops. It is also found naturally in fruits.

Carcinogenicity, International Agency for Research on Cancer (IARC) category

a. IARC Carcinogenicity Ratings for CAS109-94-4 (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2010;
b. This agent has shown little or no tumorigenic potential
c. At the time of the above review, no data was available to assess the mutagenic or genotoxic potential of this agent.

The National Center for Biotechnology Information (2021) states that ethyl formate can interact with the environment via the compound's manufacturing, transportation, removal, its use as a flavouring agent, and as an insecticide for grain and dried fruit; and that if dispersed into soil, it is expected to volatilise from the surface and promptly seep into the soil; EF will then rapidly biodegrade and chemically hydrolyse, particularly in soil with a high pH due to its high Henry's law constant. The authors also state that a volatilisation half-life of 4.5 hours (hrs) was predicted for ethyl formate from a model river; the compound isn't expected to sorb to sediment or bioconcentrate in aquatic organisms; hydrolysis is a critical fate process, particularly in waters with a high pH (half-life is 3.1 days and 7.5 hrs at pH 7 and 8, respectively); EF will react with hydroxyl radicals in the atmosphere, with an estimated half-life of 11 days; EF is water soluble, with rain able to easily dilute the compound from the air; and that typical routes for exposure are the ingestion of contaminated food, and occupationally via inhalation and dermal contact (National Centre for Biotechnology Information, 2021).

1.5. Ethyl formate toxicity to pests and mode of action

The mechanisms of toxicity of many fumigants are still in research around the world. The toxicity of ethyl formate toward insects may be due to formate inhibiting the respiratory electron transport chain enzyme cytochrome-c-oxidase, resulting in chemical asphyxiation of the insect's cells, and ultimately death (Haritos and Dojchinov, 2003). When ethyl formate goes into the body of insects, it gets broken down to ethanol and formic acid (Haritos et al., 2000). Previously, Haritos and Dojchinov (2003) determined that formate esters, particularly ethyl formate, exert toxicity after they are rapidly metabolised to formic acid in vivo in the stored product pest *Sitophilus oryzae*. The work of Haritos and Dojchinov (2003) additionally showed substantial formic acid liberation from ethyl-formate in vitro by beetle homogenates. The insect's rate of respiration is a key factor in in determining ethyl formate's toxicity, with a faster rate increasing susceptibility, and a slower rate decreasing susceptibility (Haritos, 2005).

1.6. Reactivity of ethyl formate and residue in food

As previously stated, ethyl formate reacts under neutral conditions (non-acid or non-alkaline) with water to form formic acid and ethanol, a well-known hydrolysis reaction (Mata-Segreda, 2000; Morrison and Boyd, 1992). Mata-Segreda (2000) illustrate that this reaction proceeds to an un-stated equilibrium ratio in excess liquid water at 30 °C in approximately 4-5 hours, at 50 °C in 1 hour; the reaction being very temperature dependent. Further, the hydrolysis is reported to proceed much faster when catalysed by acidic or alkaline conditions (Newling and Hinshelwood, 1936; Shah and Amis, 1954).

Other researchers have claimed that ethyl formate is very stable in distilled water (Ghittori et al., 1984). The reaction equilibrium constants (K_c) of liquid-based hydrolysis of ethyl formate were measured at 25 °C for dilute concentrations of ethyl formate in water of K_c=0.03 and

0.009 mol (EF)/mol (H_2O) with 0.025 mol/L of HCl catalyst to speed up the reaction rates to be 0.38 K_c and 0.40 K_c respectively (Mai, 2006). These values were confirmed by measuring the reaction equilibrium constants of the reverse esterification reaction of ethanol with formic acid with values of K_c=1/2.6=0.38 (Mai, 2006). Reaction equilibrium constants of this magnitude indicate that the reaction of ethyl formate in water will proceed such that less than half the ethyl formate available will react to form ethanol and formic acid, (roughly 30 mol % for equivalent molar concentrations of reactants; EF and H_2O).

Hydrolysis is "thought to occur for ethyl formate adsorbed by grain although more rapidly" (Haritos et al., 2003); however specific evidence demonstrating that grain-based hydrolysis occurs was not provided.

Haritos et al., (1999) found the concentration of liquid ethyl formate when directly applied to wheat within a sealed 50 tonne silo at a rate of 90 g/tonne, were not distinguishable from the EF concentration found on untreated control wheat samples when removed following a four-week exposure. Reuss and Annis (2003) discussed the formation of ethanol during the adsorption of ethyl formate by a range of rice fractions but concluded that the formation of ethanol in rice products could not be distinguished from that occurring in pure air (i.e. the control experimental run).

Natural levels of ethyl formate have been measured in wheat (Desmarchelier et al., 1998) at levels of ≦1 mg/kg, wheat and barley at 0.02 to 1 mg/kg respectively (Desmarchelier et al., 1999) and grains (wheat, barley, oats and canola) at levels of 1 to3 mg/kg (Ren and Desmarchelier, 2002). Natural levels of the ethyl formate hydrolysis reaction product formic acid were measured at relatively high levels of 197 to 243±22 mg/kg for wheat and 237±48 mg/kg for barley (Desmarchelier et al., 1999).

The hydrolysis reaction product ethanol occurs naturally at much lower levels of 0.006±0.009 mg/kg for wheat and <0.01 mg/kg for barley (Desmarchelier et al., 1999). Fruits naturally produce volatile compounds that are important for aromatic and flavor characteristics (Nursten, 1970). Plant volatiles such as ethyl formate have been shown to have insecticidal properties (Vincent and Lindgren. 1971; Rohitha et al., 1993).

Haritos et al. (2003) argued that the hydrolysis of ethyl formate with water held in wheat kernels will be substantially slower than that of pure water due to the physical restriction of the available water reactant. Furthermore, the hydrolysis reaction equilibrium characteristic indicates that hydrolysis will not proceed to completion such that all the ethyl formate is reacted to hydrolysis products. But the hydrolysis reaction rate in grain cannot be estimated or predicted based on available information, nor has it been measured.

The fact that relatively large quantities of ethyl formate are adsorbed from air, but the presence of pure ethyl formate residues in "aged" fumigated grain has been found to be minor/negligible, implies that the ethyl formate reacts in the grain or desorbs through reaction or dissipation in air. However, most methods used to extract ethyl formate from grain that had been dosed with ethyl formate by gaseous or liquid means and aged for many weeks (ethyl formate residues present in grain for many weeks), usually involved aqueous alcohol or pure water extraction solvents for up to 48 hours (Haritos et al., 2006). So, any ethyl formate extracted is expected to undergo hydrolysis in the excess water during this extraction period, preventing the measurement of pure ethyl formate present.

1.7. Reactivity of ethyl formate with materials

There have been limited assessments in literature concerning ethyl formate's reactivity with materials encountered in fumigations. One study however, conducted by Rajendran (2001) evaluated the inter-reaction between ethyl formate when used as fumigant, and unpainted iron

and steel. The results of this study reported that ethyl formate, when degraded to ethanol and formic acid, was capable of corroding unpainted iron and steel (Rajendran, 2001).

1.8. Application technology of ethyl formate

Fumigants, such as hydrogen cyanide and methyl bromide, are discharged from cylinders or other containers placed outside the chamber (Bond and Monro, 1984). When hydrogen cyanide is dispersed into a fumigation chamber, further pressure on the cylinder is required (Bond and Monro, 1984). This can be done via the use of an air compressor pump.

Ethyl formate has been distributed as a cylinderised gas commercially as Vapormate®. Vapormate® is a non-flammable product (BOC PCT/AU03/00087) containing 16.7 weight (wt.) % ethyl formate dissolved in liquid carbon dioxide (CO_2), which acts as a propellant (Lawrence, 2005). In field trials fumigating grain silos using forced-flow techniques, Vapormate® has been circulated via the use of a high-powered industrial fan (Lawrence, 2005). The use of the fan reduces the residence time of fumigant near grain, limiting sorption and providing an even distribution of ethyl formate. By ensuring an even distribution of fumigant, it allows for a reduction in the amount of ethyl formate required for an efficacious treatment, resulting in decreased residual fumigant in the grain post fumigation. The flammability of ethyl formate is significantly reduced by the presence of high concentrations of carbon dioxide in Vapormate® (Ryan and Bishop, 2003). Pure ethyl formate has a lower explosive limit of 2.6% and an upper explosive limit of 18.2%, providing substantial flammability risk as a fumigant (Ryan and Bishop, 2003). Storage and delivery of ethyl formate from a cylinder also limits handling and exposure risks to the applicator (Haritos et al., 2003).

Liquid fumigants are stored at room temperature prior to fumigation. For fumigation treatments, liquid ethyl formate can be poured into a shallow tray inside the chamber; or onto burlap sacks or filter paper acting as an intermediary, stopping the solvent from making direct

contact with the commodity (Bond and Monro, 1984). The intermediary material also enlarges the surface area of the solvent, subsequently increasing the solvent's rate of evaporation (Xin et al., 2008). Once the chamber is sealed, industrial fans are started, circulating air across the liquid or the stacked material increasing evaporation and evenly distributing the fumigant (Bond, 1984).

1.9. Stored product insects

Rhyzopertha dominica (Fabricius), commonly known as the lesser grain borer (Figure 1-1) is a primary grain insect pest belonging to the Bostrichidae family (Walker, 2006). This species is an external feeder, only feeding from the outside of the grain kernel (Davis, 2003). All emergent life stages (adults, pupae, larvae) have been documented to feed and burrow into grain kernels, with females capable of laying several hundred eggs during their lifespan (Campbell et al., 2004). Eggs are laid externally to the individual grain kernels, whereupon larvae emerge from the egg, and burrow into the grain (Campbell et al., 2004). Because of *R. dominica* feeding on the grain, significant damage is inflicted; resulting in partial destruction of the kernel, potentially toxic residue, and grain susceptibility to pathogens (Campbell et al., 2004).

Figure 1-1. Lesser grain borer adults attacking kernels of grain (Australian Museum, 2020).

Tribolium castaneum (Herbst), commonly known as the rust red flour beetle (Figure 1-2) is an insect pest belonging to the Tenebrionidae family (Walker, 2006). As a secondary

pest, *T. castaneum* is unable to attack grain kernels, instead targeting stored grain cereal and other processed grain products (Department of Primary Industries and Regional Development, 2018). Females are capable of laying up to 400 eggs throughout their lifespan. Once larvae have emerged, they join mature insects to feed. The life cycle takes four to eight weeks and adults may live as long as 12 months (Department of Primary Industries and Regional Development, 2018).

Figure 1-2. Rust red flour beetle adult attacking kernels of grain (Emery and Cousins, 2019).

Sitophilus oryzae (Linnaeus), commonly referred to as the rice weevil (Figure 1-3) is a major insect pest of whole cereal grains belonging to the Curculionidae family (Walker, 2006). Classified as an internal feeder, larvae of *S. oryzae* feed entirely within the grain kernel (Davis, 2003). Females gnaw into the kernel, making a hole just large enough to lay an egg into, before sealing the hole with a gelatinous secretion (Department of Primary Industries and Regional Development, 2018). Upon hatching from the egg, larvae feed on the kernel until pupation, finally eating their way out as a mature insect (Department of Primary Industries and Regional Development, 2018). The development from egg to adult life takes approximately four to six weeks with a lifespan of up to eight months (Department of Primary Industries and Regional Development, 2018).

Figure 1-3. Rice weevil adult attacking kernels of grain (Berger, 2020).

1.9.1. Toxicity of ethyl formate to stored grain insect pests

The toxicity of ethyl formate towards common stored grain insect pests (*R. dominica*, *T. castaneum*, and *S. oryzae*) has been extensively studied via laboratory bioassays described in Muthu et al. (1984), Damcevski et al. (2001), Damcevski, (2006), Wright et al., (2001) and Damcevski and Annis (2001). The aforementioned studies found that ethyl formate was effective in achieving mortality (> 95%) against all life stages of *R. dominica*, *T. castaneum*, and *S. oryzae*. The results of these studies is reported in Table 1-3, which identified key gaps in the research to be addressed. The principal research gap identified was that ethyl formate needed to be evaluated on a larger scale to accurately simulate the effectiveness of the fumigant towards insect pests in real-world grain storage conditions.

Table 1-3. Toxicity of ethyl formate against stored grain insect pests

Target Insect	Stages	Type of Commodity. & Filling Ratio (%)	Moisture Content or Relative Humidity (%)	Exposure Time	Temperature (°C)	Dose (mg/L or g/t)	*Ct* Product (mg h/L)	Mortality (%)	Reference
S. oryzae	Egg		60-80	24hr	27.4±2		283	95	Muthu et al., 1984.
S. oryzae	Larvae		60-80	24hr	27.4±2	14.8-27.1	384–727	95	
S. oryzae	Pupae		60-80	24hr	27.4±2	44.0	1110	95	
S. oryzae	Adult		60-80	24hr	27.4±2	19.2	588	95	
T. castaneum	Egg		60-80	24hr	27.4±2	5.3	154	95	
T. castaneum	Larvae		60-80	24hr	27.4±2	17.4	590	95	
T. castaneum	Pupae		60-80	24hr	27.4±2	27.9	669	95	
T. castaneum	Adult		60-80	24hr	27.4±2	22.3	643	95	
S. oryzae	Adult	Wh. 50	60	12min	25	340		100	Damcevski et al., 2001.
S. oryzae	Adult	Wh. 50	60	2hr	25	210		100	
S. oryzae	Adult	Wh. 50	60	3hr	25	130		94	
S. oryzae	Larvae	Wh. 20	60	48hr	25	109		100	
S. oryzae	Larvae	Wh. 50	60	48hr	25	130		100	
S. oryzae	Larvae	Wh. 80	60	48hr	25	155		100	
S. oryzae	Adult		100	24hr	25	14	304	99.9	Damcevski, 2006
S. oryzae	Adult		30-50	24hr	25	30	660	99.4	
	Adult					36.4	790	99.9	
S. oryzae	Larvae+early pupae	0.3	30-50		25		660	100	Wright et al., 2001
S. oryzae	Larvae+early pupae	0.3	100		25		300	99.9	
S. oryzae	Adult	0.3	55	24hr	25-30		1500	100	Damcevski, 2006
S. oryzae	Mid pupae	0.3	55	24hr	15		>2200	100	
R. dominica	All	0.3	55	24hr	25-30		1300-2200	100	
T. castaneum	Larvae	0.3	55	24hr	15		1300	100	
					25		1500	99	
					30		1700	99	
R. dominica	All stages		55	24hr	29	70		99	Damcevski and Annis,
T. castaneum					24	66			
S. oryzae					16	60			2001
S. oryzae	Pupae	48	12%	48hr	25	300		99	Damcevski, 2006

1.10. Brown Marmorated Stink Bug

Halyomorpha halys (Stål), commonly known as the Brown Marmorated Stink Bug (BMSB) is a polyphagous insect capable of feeding upon over 300 species of plants, ranging from fruit and vegetables to native vegetation (Lee et al., 2013). It also attacks a wide range of ornamental plants, and can be a household nuisance during winter months when the bugs seek shelter indoors (Center for Agricultural Bioscience International, 2014). The adjective 'marmorated' in its common name refers to the insect's marbled or mottled dorsal coloration, although individual coloration can be quite varied (Center for Agricultural Bioscience International, 2014). The BMSB is native to northeast Asia but can be found all over the world (Valentin et al., 2017). A contributing factor to this has been the insect's ability to reproduce in a variety of climatic conditions ranging from 17 °C to 33 °C; enhancing its survivability whether that be in Asia, Europe or North America (Nielsen et al., 2008).

Figure 1-4. Brown marmorated stink bug adults attacking crab apple leaves (New Jersey Agricultural Experimental Station, 2019).

The BMSB has five developmental phases/instars with development from egg to adult taking 538-degree days, or 32-35 days at 30 °C (Nielsen et al., 2008). However, given its developmental threshold range between 17 °C and 33 °C, insects typically develop to adults

between 33-45 days (Nielsen et al., 2008). The developmental cycle is initiated when females lay eggs on the underbelly of leaves in clusters of 28 eggs, with the ability to lay approximately 244 eggs over their lifespan (Nielsen, 2008). Eggs initially appear light green, before changing to an opaque white colour (Rice et al., 2014). Approximately 3-6 days after the eggs have been laid, first instar insects emerge, subsequently feeding on the remaining eggshell. Second instars develop 3-5 days later and begin to feed on the plant on which their eggs were laid. Emerging after a further 3-5 days, the third instar insects are followed by fourth (6-7 days later) and fifth instars (13-14 days later) (Rice et al., 2014). Once the fifth instar has developed, it takes approximately another 7-15 days to complete the developmental cycle depending on environmental conditions (Rice et al., 2014).

Like its Pentatomidae relatives, the BMSB will predominantly damage and feed on the fruiting structures of plants; but differs by also feeding on other parts of the plant such as the leaves, stalks, stems and even seeds (McPherson and McPherson, 2000). Damage to fruit typically occurs when the BMSB pierces the fruit with their stylet to drain and feed on the plant's fluids (McPherson and McPherson, 2000). Along with the loss of plant fluids, feeding results in the deformation and decay of fruit and seeds and delayed plant maturations; leaving the plant susceptible to various pathogens (McPherson and McPherson, 2000).

The BMSB is regarded as a migratory insect that can easily move between host plants (Jentsch, 2008), migrating from plants with early-ripening fruit to those with late ripening fruit (Welty et al., 2008). As with other Pentatomidae, both mature and immature BMSB's can scatter to feed on vulnerable hosts (Tillman et al., 2009). In the event of a disturbance, the BMSB can drop off host plants or escape to sheltered areas (Bernon, 2004). Adults possess a long-range flight capability, with an approximate daily range of 2 km (Lee and Leskey, 2015). As a result of the insect's high level of mobility, it is able to infest new areas far more easily, thus further enhancing its survivability and distribution.

BMSB typically congregate in large numbers at the start of winter due to the release of an aggregation pheromone once they have found a suitable overwintering location (Khrimian et al, 2014). Two primary epoxides make up this aggregation pheromone (3S,6S,7R,10S)-10, 11-epoxy-1-bisabolen-3-ol (3) and (3R,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol (Khrimian et al, 2014). In colder climates, the aggregation pheromone results in large numbers of sexually mature BMSB, typically in the hundreds, to seek shelter inside enclosed structures such as warehouses, homes and vehicles (Watanabe, 1994; Hamilton, 2009). A study conducted by Inkley (2012) examined overwintering behaviour of the BMSB inside a home located in Maryland, USA. Over the course of 181 days, approximately 26,000 adult BMSB's were collected, displaying the significant nuisance posed towards households.

By happenstance, some of the structures infested with overwintering BMSB store vehicles, equipment and materials that are then shipped internationally; allowing the insect to hitchhike and infest new territories.

Hitchhiking BMSB could potentially enter Australia via the shipping of motor vehicles and farm machinery from infested countries within northeast Asia, North America, and mainland Europe (Hoebeke and Carter, 2003).

According to a report by the New Zealand Ministry for Primary Industries, overwintering BMSBs arriving from the northern hemisphere have the greatest probability of successful establishment (Ormsby, 2018). This is predominantly due to the warmer temperatures encountered on arrival to southern hemisphere nations such as Australia and New Zealand; acting as a stimulant to end overwintering and commence reproduction (Niva and Takeda, 2003).

A key example of the threat posed by the BMSB is its establishment in the United States. The BMSB is thought to have arrived in the United States in 1996 aboard a single shipment from

China, and is now a widespread agricultural pest, present in 42 states; including all states east of the Mississippi River, and several on the west coast (Xu et al., 2014; Hahn et al., 2016; Leskey et al., 2012). Emphasising the biosecurity threat are the findings published by Xu et al. (2014), who stated that Stink bugs native to the United States overwinter under leaf litter and debris (McPherson and McPherson, 2000) and there have been undocumented reports that BMSB may also overwinter in forest areas (Wallner et al., 2014). In the United States, BMSBs begin searching for an overwintering site at the end of September (Hoebeke and Carter, 2003). After entering an over wintering site, BMSB remain inactive in the overwinter site until the end of May in the Northeast (Hoebeke and Carter, 2003). Overwintering in protected sites is a survival strategy that minimises metabolism and allows the pest to survive during unfavourable environmental conditions until conditions improve and host plants become available (Holtz and Kamminga, 2010).

Feeding by both the adult and nymphal stink bugs has caused damage to high value economic horticultural crops in the United States. In 2009, BMSB was reported to cause economic damage to apples, peaches, cherries, tomatoes, corn, and soybeans (Leskey and Hamilton, 2010). Losses or diversion from the fresh to process market of these crops due to BMSB feeding could have significant market impacts. Numerous states have attributed BMSB damage to substantial horticultural cropping losses, including California, Delaware, Maryland, New Jersey, Oregon, Pennsylvania, Tennessee, and West Virginia (Holtz, 2010). However, reliable reports of the specific crops and level of damage is yet to be reported. Researchers at the United States Department of Agriculture-Agricultural Research Service laboratory in Kearneysville, West Virginia studying the impact of BMSB have provided the most specific reports of damage (Leskey and Hamilton, 2010). 50-60% of the stone and pome fruit grown commercially in Maryland and the eastern panhandle West Virginia were observed to be injured by BMSB, and some growers lost their entire crop (Leskey and

Hamilton, 2010). These losses appear similar to reports the researchers received from southeastern Pennsylvania and parts of New Jersey and Virginia (Leskey and Hamilton, 2010). In these areas, the population of BMSB is reported to be extremely high (Leskey and Hamilton, 2010). The level of damage appears directly correlated with the population level of BMSB, indicating that the insect has the potential to have a widespread effect on the Unites States agricultural industry throughout the country (Holtz and Kamminga, 2010). Currently, there appears to be few environmental limiting factors for BMSB populations. (Holtz and Kamminga, 2010)

H. halys has become the dominant Pentatomid species in many mid-Atlantic areas (Nielsen and Hamilton, 2009; Nielsen et al., 2011), causing $37 million loss to apples in 2010 (Seetin, 2011), and some stone fruit crops losing 90% of their yield (Leskey and Hamilton, 2010). Moreover, great potential for similar economic losses exists in southern, western and mid-western states (Holtz and Kamminga, 2010).

The BMSB infestation of the United States has had several flow-on effects, particularly relating to the use of broad-spectrum insecticides for their control. The insecticides nonspecific targeting resulted in the mortality of numerous other insects which were natural predators to typically controlled pest species; allowing the European red mite and wooly apple aphid population to increase significantly and damage crops (Leskey et al., 2012).

1.11. Aims of this thesis

Chapter 1: outlined that fumigation and controlled atmosphere is the primary treatment modality for post-harvest and quarantine / biosecurity. This chapter also identified the substantial need for a new fumigant to replace those currently used due to their negative environmental and human health effects. Ethyl formate is proposed in this thesis as being a

potential fumigant for two key applications: post-harvest protection of stored/durable commodities, and quarantine/biosecurity treatments.

However, in order for ethyl formate to be considered for these applications, it is vital that the following assessments are conducted:

- **Chapter 2**: Determine the financial risk posed by successful establishment in Australia of the BMSB on a small agricultural industry; Assess the cost effectiveness of ethyl formate as a quarantine / biosecurity treatment.
- **Chapter 3**: Evaluate the efficacy of ethyl formate as a quarantine / biosecurity treatment for management of the BMSB.
- **Chapter 4**: Assess the efficacy of ethyl formate as a post-harvest treatment for controlling insect pests effecting a large agricultural industry.
- **Chapter 5**: Assess suitability for ethyl formate use in proximity to various materials and vulnerable electronic componentry.

Chapter 2: Wine industry risk analysis for the Brown Marmorated Stink Bug with a focus on mitigation

2.1. Introduction

The brown marmorated stink bug, *Halyomorpha halys*, (Hemiptera: Pentatomidae) (BMSB), is an agricultural and household pest known to attack over 300 types of plant, including grapes, citrus, stone fruit, berries, asparagus, soybean and corn (Centre for Agricultural Bioscience International, 2014). The insect is an identified biosecurity pest for Australia, with its primary pathway of entry via hitchhiking on cargo shipped into the country (Commonwealth of Australia, 2019).

From its native range in Northeast Asia, BMSB has now spread to North America and Europe (Switzerland, Germany, France, Hungary, Greece, Italy) (Cesari et al., 2015).). In Australia the insect was first detected in December 2017 at Glendenning in Western Sydney, NSW in a shipping container, triggering an eradication response (New South Wales Department of Primary Industries, 2017). A second detection occurred in January 2018 at Horsley Park, also in Western Sydney (AusVeg, 2018). In February 2018 the insect was detected in Jandakot, WA, in electrical products imported from Italy (AusVeg, 2018). BMSB was successfully eradicated from Australia.

However, the BMSB threat remains, and between September 1 and April 30 of each year the Australian Department of Agriculture, Water and the Environment (DAWE) implements strict biosecurity measures against inbound freight from 37 countries classed as high risk for BMSB (Department of Agriculture, Water and the Environment, 2021a).

As a result, it was deemed important to firstly explore the financial threat posed by the BMSB on the Australian agricultural industry in the event of a major outbreak. The Australian wine industry was selected as a case study, due to its substantial economic value ($40 billion annually) and the BMSB threat faced via the importation of barrels, winemaking equipment and machinery from high-risk European countries such as France, Italy, Hungary

and Germany (Wine Australia, 2022; Australian Chief Plant Protection Office, 2020). BMSB has been documented to damage mid Atlantic vineyards in the United States resulting in decreased grape quality and yield (Leskey et al., 2012). BMSB have also been known to cause grape contamination due to their release of stress compounds which taint the wine, and compromise product quality (Mohekar et al., 2017).

To protect their vines, growers have resorted to applying broad spectrum insecticides to ensure sufficient insect control; however, the vine canopy may reduce spray coverage, reducing the chemical's effectiveness (Pfeiffer et al., 2012). Broad spectrum insecticide use may also result in beneficial insect mortality, leading to secondary pest problems such as increased activity of mealybugs and other disease vectors (Pfeiffer et al., 2012). For organic and biodynamic growers, the use of insecticides is heavily restricted, further compounding the extent of BMSB damage in the vineyard.

The financial risk analysis performed in this study involved an estimate of the likely loss reduction to the Australian wine industry by eradicating BMSB outbreaks was conducted. The financial threat of BMSB to wine grape growers was assessed on the basis of yield loss, avoided foliar applications of insecticide, labour and machinery costs, and monitoring costs. A simple bioeconomic model was used to simulate the spread and resultant costs of BMSB to the wine industry over a 30-year timeframe.

To complement the risk analysis, this study also sought to evaluate the cost effectiveness of biosecurity treatments used to protect Australia from BMSB infestation.

2.2. Materials and Methods

2.2.1. Financial threat posed by BMSB to the Australian wine industry

The bioeconomic model used to assess the financial threat posed by BMSB was derived from that described in Fraser et al. (2019). This model was chosen due to its simplicity in simulating the spread of BMSB with a sigmoid curve. Due to the absence of accurate spread data to fit a curve to, and the lack of parameter estimates from field observations such as mean dispersal distance, it was not possible to use a reaction diffusion model.

All BMSB costs quantified in the input model relate to agricultural damages across n wine production regions within Australia. They do not include environmental impacts or effects on households. Damage costs, d, are estimated in a nil management scenario (d^{NM}) whereby there is no government response effort to eradicate an incursion. All costs used for this analysis, and all results are in Australian dollar (AUD).

For a BMSB host grape variety j, the model assumes that a spatially homogenous environment exists through which the insect will spread once it has become established. The area of a host affected by BMSB is simulated using a logistic growth equation where the proportion of a host j in region i affected in period t, a_{ijt}, increases over time following Equation 2-1:

$$a_{ijt} = a_{ij}^{\max} \frac{r_{ij}^{\max}}{1+\left(\frac{r_{ij}^{\max}}{r^{\min}}-1\right)e^{-\omega t}} \qquad \text{Equation 2-1}$$

Here, $a_{ij}^{\max}$ is the maximum area of host j in region i than can be affected; $r_{ij}^{\max}$ is the maximum proportion of host j affected by BMSB at in region i; $r^{\min}$ is the initial proportion of hosts affected upon BMSB establishment (assumed constant for all hosts in all regions), and ω is an infestation growth rate (assumed constant across hosts and regions).

Equation 2-1 states that the total area of host crops affected in region i increases at an increasing and then a decreasing rate over time, and that the speed of spread is dictated by the growth parameter, ω. In the absence of information about ω, a hypothetical infestation growth is determined by the number of periods it takes BMSB to affect a given proportion (θ) of $a_{ij}^{\max}$ such that:

$$\omega = -t_{\theta}^{-1} \ln \left[\frac{r_{ij}^{\max} - \theta}{\theta \left(\frac{r_{ij}^{\max}}{r^{\min}} - 1 \right)} \right] \qquad \text{Equation 2-2}$$

Here, θ is a specified proportion of $a_{ij}^{\max}$ infested and t^{θ} is the number of periods taken for BMSB to reach the infestation level θ.

The predicted area affected by BMSB in region i in period t (A_{it}) across n host crops is:

$$A_{it} = \begin{cases} \sum_{j=1}^{n} a_{ijt} \text{ if } A_{it} < \sum_{j=1}^{n} a_{ij}^{\max} \\ \sum_{j=1}^{n} a_{ij}^{\max} \text{ if } A_{it} \geq a_{ij}^{\max} \end{cases} \qquad \text{Equation 2-3}$$

Equation 3 is a piecewise function defined on two intervals, $a_{it} < \sum_{j=1}^{n} a_{ij}^{\max}$ and $a_{it} \geq \sum_{j=1}^{n} a_{ij}^{\max}$. It states that the area affected by BMSB over a specified period equals the summed area of each host crop affected.

The damage cost of BMSB in a region i containing j hosts in period t under the nil management policy (d_{it}^{NM}) is calculated in the model as:

$$d_{it}^{\mathrm{NM}} = \sum_{j=1}^{n} \left(V_{jt} + YP_{jt_0} \right) A_{ijt} \qquad \text{Equation 2-4}$$

where: V_{jt} is the increase in variable cost of production per ha induced by BMSB management in year t (assumed constant across regions); Y is the mean change in host yield resulting from BMSB infestations (assumed constant across host grape varieties, regions and

time); P_{jt_0} is the world price for host variety j in year t_0; and A_{ijt} is the area of host j affected by BMSB in region i in year t.

Equation 2-4 states that in the nil management scenario BMSB causes yield reductions and chemical costs increases in host crops, and that both of these costs increase as the area affected by the insect increases. Variable costs consist of additional chemical, labour and machinery costs for chemical control measures and additional monitoring costs (Table 2-1).

Note that for simplicity it is assumed Australia is a price taker with respect to host commodities such that the damage caused by BMSB is not sufficient to place significant upwards pressure on host prices.

Table 2-1. Increased variable production costs per year attributable to BMSB.

Parameter	**Assumed value (AUD$/ha)**
Increased insecticide costs[a]	12.50
Labour and machinery costs[b]	100.00
Machinery costs[c]	100.00
Monitoring costs[d]	200.00

[a] Representative cost based on indoxacarb ($366.66 /kg (HerbiGuide, 2020)) applied two additional times at 17 g/100 L water (Fisher et al., 2019);

[b] Labour cost assumes a casual wage rate of $50 /hr, time per application of one hour/hectare and a total of two additional applications. The sensitivity of results to the number of applications is tested in the results section, but a base case of two applications was chosen as a plausible estimate given chemical withholding periods. In the case of the representative chemical used for a cost estimate (indoxacarb), the withholding period is 56 days (Fisher et al., 2019);

[c] Spray rig costs (fuel, oil and maintenance) of $50 /hour, time per application of 1 hour/hectare and a total of two additional applications.

[d] Labour cost assumes a casual wage rate of $50 /hour and an additional time per hectare invested in trap maintenance, lure rotation (i.e. BMSB is attracted to different stimuli at different times of the year (Leskey et al., 2015)) and monitoring of three hours/year. Costs of insect traps and lures are assumed at $50 (Morrison et al., 2015), with traps deployed at one trap per hectare for monitoring purposes.

Costs may be associated with wine taint resulting from BMSB being crushed with grapes. The odorants released by the insect are unstable compounds that break down easily, and so have a negligible impact on flavour (Baldwin et al., 2014, Fiola, 2011). However, relatively high concentrations of one of the compounds produced by distressed BMSB, known as trans-2-Decenal, has been found to affect the taste of Pinot noir (Mohekar et al., 2017), chemical suppression of the pest is assumed to maintain population levels at levels where taint does not occur.

Since d^{NM} accrues over time, it is subject to discounting. Discounting has an erosive effect on monetary values that increases with time, meaning that the value of one unit of damage caused in the present is worth more in real terms than the same unit of damage caused in the future.

Applying discounting, the present value of damage anticipated from BMSB over t periods ($\mathrm{PV}_t^{\mathrm{BMSB}}$) is the sum of d_{it}^{NM} across all affected regions (n) in Australia (Equation 2-5) where v is the discount rate.

$$\mathbf{PV}_{\boldsymbol{t}}^{\mathbf{BMSB}} = \sum_{i=1}^{n} \left[\frac{d_{it}^{\mathrm{NM}}}{(1+v)^t} \right] \qquad \text{Equation 2-5}$$

In the results section to follow, $\mathrm{PV}^{\mathrm{BMSB}}$ is given for a 30-year infestation period where the initial incursion occurs in year one. Model parameters and their assumed values appear in Table 2-2.

Table 2-2. Parameters used to assess the spread and impact of BMSB.

Parameter	**Assumed value**
Area initially affected, $A^{\min}$ (hectare).[a]	1.00
Discount rate, υ (%).[b]	7.00
Increased variable cost of production, V_{it} ($/ha).	See Table 5-1
Maximum area affected, $a_{ij}^{\max}$ (ha).[c]	124 700
Maximum proportion of host affected, $r_{ij}^{\max}$(%).[a]	95.00
Minimum proportion of host affected, $r^{\min}$(%).[a]	0.01
Prevailing price for affected commodities at $t = 0$, P_{it_0} ($/Tonne).[e]	700.00
Proportion of host infected at t^{θ}, θ (%).[a]	90.00
Time taken for θ of host to be infected (year).[a]	15.00
Yield reduction despite control, Y (%).[a]	10.00

[a] Plausible estimate.

[b] Commonwealth of Australia (2006).

[c] Australian Bureau of Statistics (2021a).

[d] Australian Bureau of Statistics (2021a), Australian Bureau of Statistics (2021b).

2.2.2. Cost effectiveness of biosecurity treatments

A review of available literature on the currently implemented biosecurity treatments was conducted, looking at treatment efficacy as well as drawbacks (Webley, 2012; Kuhar and

Aigner, 2016; Associated Customs and Forwarding, 2019; Department of Agriculture, Water and the Environment, 2021ab; Ormsby, 2018; Park et al., 2020; Baur et al., 2015; Svedberg and Johanson, 2013; Bell, 2006; Johnson et al., 2012; United States Environmental Protection Agency, 2021; Papadimitriou et al., 2008; Muhle, 2009; Shipping Australia Limited, 2019; National Center for Biotechnology Information, 2021; Krishna et al., 2005; Ryan and De Lima, 2014; Muthu et al., 1984; Ren and Desmarchelier, 2002; Hilton and Banks, 1997).

Collaboration with shipping and biosecurity industry contacts provided approximate costings for the reviewed biosecurity treatments (Australian Chief Plant Protection Office, 2020; Page, R., personal communication, 21/06/20221; Schelfhout, J., personal communication, 23/06/2021; Short, J., personal communication, 15/06/2021; Luckens Fumigation Services, 2020).

Information on the approximate volume and location of offshore biosecurity treatments was provided via a collaborative relationship with the Australian Chief Plant Protection Office (as part of DAWE). The information provided was in the form of submitted pre-shipment treatment certificates from approved offshore treatment providers.

2.3. Results

2.3.1. Financial threat posed by BMSB to the Australian wine industry

Results below indicate BMSB could cause over $45 million of damage to the wine industry per year, equivalent to a 5% contraction of the industry. The social impacts of the insect on households are not included but given their severity in other parts of the world they are an important consideration when choosing a management policy for this insect.

Figure 2-1 depicts the area growth (i.e. A_{it}, Equation 2-3) and damage cost to the Australian wine industry expected from BMSB over time under a nil management scenario. The spread

model assumes that 90% of the 250,000 hectare of wine grapes in Australia could be affected after 15 years.

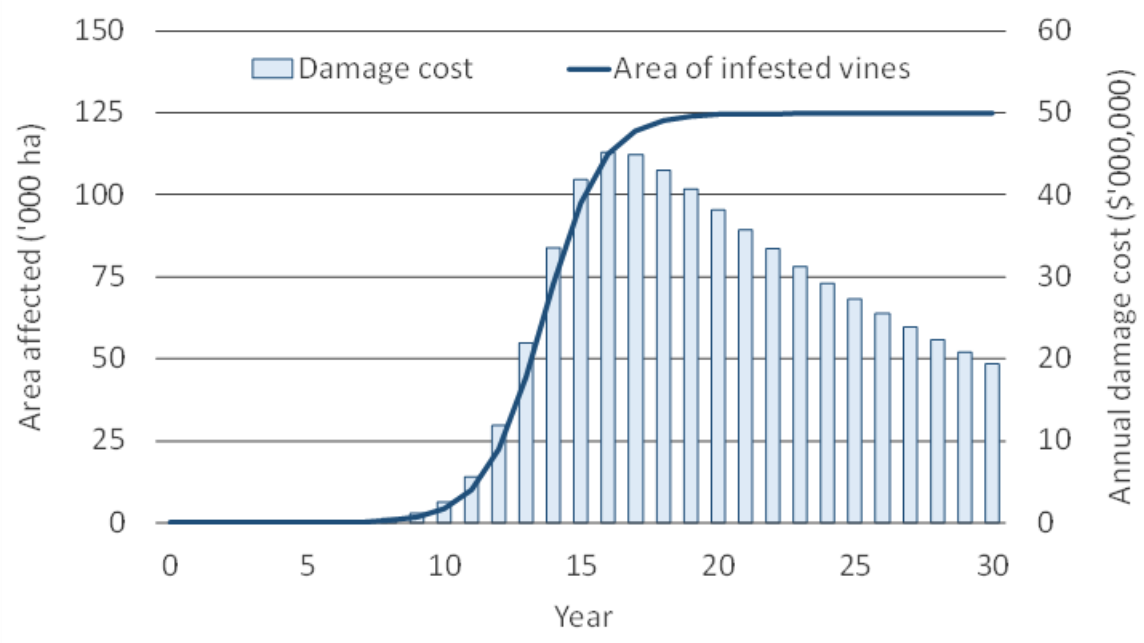

Figure 2-1. Predicted area of wine grapes affected by BMSB in Australia over 30 years and resultant damage costs per year.

The area infested is likely to be relatively small until approximately 10 years after infestation. At this point the population increases rapidly, affecting 90% of the production area by year 15. Damage costs follow the same pattern, with annual damage costs peaking at $45.1 million in year 16. Thereafter, the effects of the discount rate erode annual damage costs to $18.9 million by year 30 of the simulation.

To measure the effect of parameter values on model output, each parameter is varied across a specified range while holding all other parameters constant (Figure 2-2). Here the seven parameters producing the most change are ranked from top to bottom according to their strength of influence on the present value of BMSB damage over 30 years. Parameters in Figure 2-2 are varied over plausible ranges (discount rate 5-9%, yield loss 5-15%, maximum

proportion of vines affected 80-100%, number of additional sprays 1-3, chemical cost $5.00-7.50, monitoring costs $100-300, time taken for 90% of vines to be affected 10-20 years).

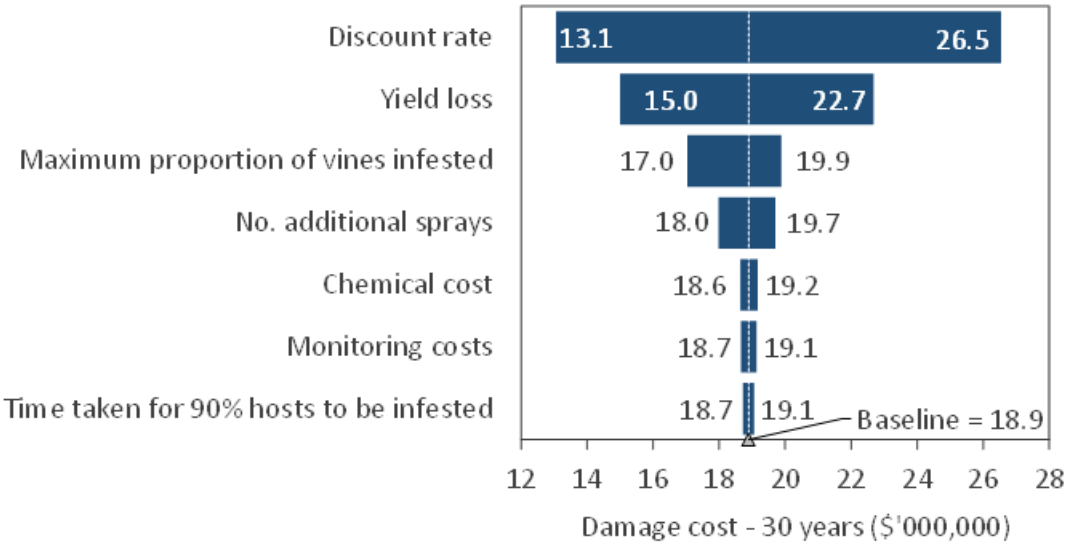

Figure 2-2. Sensitivity analysis. The length of the bars corresponding to each parameter indicate how much the mean present value of damage caused by BMSB over 30 years is affected by changes in the parameter.

2.3.2. Cost effectiveness of biosecurity treatments

Biosecurity treatments of high-risk cargo are conducted offshore by approved providers, who submit a certificate to DAWE for each treatment conducted. For the 2019-20 BMSB season, approximately 68,500 offshore treatment certificates were submitted to DAWE from approved overseas treatment providers (Table 2-3). The percentage breakdown of these treatments consisted of 26% heat treatment, 29% methyl bromide and 45% sulfuryl fluoride (Australian Chief Plant Protection Office, 2020).

Table 2-3. Offshore treatment provider countries and number of treatments conducted (Australian Chief Plant Protection Office, 2020).

Biosecurity Treatment Location	Approximate Number of Biosecurity Treatments
USA	18,000
Belgium	11,000
Italy	9,000
Singapore	8,600
Germany	8,000
Turkey	4,500
Spain	3,500
France	2,800
Netherlands	2,000
Malaysia	1,100

The approximate costs (AUD) for offshore (outside of Australia) biosecurity treatments per 20-foot container are:

- USA (sulfuryl fluoride) $1300; (Page, R., personal communication, 21/06/20221;).
- Belgium (sulfuryl fluoride) $920; (Page, R., personal communication, 21/06/20221;).
- Germany (sulfuryl fluoride) $1800; (Schelfhout, J., personal communication, 23/06/2021).
- Singapore (methyl bromide) $1600; (Page, R., personal communication, 21/06/20221;).
- France (heat treatment) $500 (Page, R., personal communication, 21/06/20221;).

The approximate costs (AUD) as of 2021 for onshore (within Australia) biosecurity treatments per 20-foot container are:

- $450 for sulfuryl fluoride (Luckens Fumigation Services, 2020).
- $485 for methyl bromide (Luckens Fumigation Services, 2020).
- $550 for ethyl formate (Short, J., personal communication, 15/06/2021).

2.4. Discussion

The analysis shows that BMSB establishment has the potential to financially cripple the Australian wine industry. The annual cost predictions of the model suggest cumulative damage costs will be $7.3 million over 10 years, $252.2 million over 20 years and $318.7 million over 30 years. Figure 2-1 showed that damage is negligible for the first 8-10 years before the BMSB population increases rapidly, reaching a new steady state after year 20.

Damage costs are also relatively minor until the 8 to 10-year mark, corresponding to the inflexion point of the population curve. Between years 10 and 15 damage costs rapidly increase from approximately $5.5 million in year 10 to $41.9 million in year 15. This is despite the erosive effects of the discount rate, which are more than offset by the rate of population increase. Damage costs peak in year 16 at $45.1 million. At this point, as the spread rate effectively falls to zero (i.e. all vines having become infested), the effects of the discount rate lower the present value of damage costs to approximately $18.9 million by year 30 of the simulation.

Financial modelling results are most sensitive to changes in the discount rate, which is inversely related to the damage cost. Lowering the discount rate from its most likely value of 7% to 5% (a change of -29%) increases the present value of costs in year 30 by 40% (from $18.9 million to $26.5 million) and increasing it to 9% (a change of +29%) lowers the present value of benefit by approximately 31% (to $13.1 million). Determining an appropriate discount rate is an important and controversial issue since as it has a major impact on the viability of many public projects (Abelson and Dalton, 2018). To date there is no definitive answer as to what rate should be applied in different circumstances.

The damage parameter is also sensitive to changes in the yield loss despite the control parameter. It has a positive relationship with the damage cost. Reducing its value from the

median value of 10% to 5% lowers the 30-year damage cost from $18.9 million to $15.0 million (-21%) and increasing its value to 15% raises the damage cost to $22.7 million (+20%). This high sensitivity indicates further research is needed to better estimate the effectiveness of controls in vineyards. The range of possible yield effects from BMSB on vines used in the sensitivity analysis assumes that chemical control is relatively effective, reducing losses from the 30-47% observed in untreated vines (Smith et al., 2014).

The remaining parameters demonstrate moderate to minor sensitivity. The proportion of grape varieties susceptible to BMSB attack is assumed to be high (95%, Table 2-2), and although a proportion as low as 80% was tested, this is still a relatively high proportion. Similarly, the number of chemical applications required in addition to normal management practice was only tested between the bounds of one to three applications. Hence, these parameters tend to have a moderate effect on results across their assumed ranges.

Chemical costs and the spread rate (i.e. time taken for 90% hosts to be infested) also had relatively small ranges of plausible values ($5.00-7.50 and 10-20 years, respectively), and so produced a minor effect on 30-year damage costs. Monitoring costs were tested across a range of $100-300 per hectare per year, but also produced a minor effect on damage costs over 30 years compared to other model parameters.

The currently employed biosecurity treatments to prevent BMSB infesting Australia provide varying levels of efficacy and consistency, along with negative environmental and occupational health effects.

Heat treatment accounts for 26% of offshore biosecurity treatments. modality for the control Heat for the treatment of containers can be generated via oil or gas heaters; with fans used to ensure an even distribution throughout the container (Webley, 2012). DAWE mandates that for the control of BMSB, the coldest internal temperatures of containers must be at least 50

°C for 20 minutes. This is supported by research from Kuhar and Aigner (2016), who found that exposing adult BMSB to 50 °C heat for 15 minutes was capable of achieving 100% mortality. However, during the 2018-19 BMSB season, three treatment providers in Italy were suspended by DAWE due to live insects being detected upon arrival in Australia (Associated Customs and Forwarding, 2019). In December 2020, an additional two treatment providers in Italy were suspended due to repeated detections of live BMSB following heat treatments (Department of Agriculture, Water and the Environment, 2021b). A key factor in these failures was the inability to meet the treatment requirement that the entire container be subjected to 56 °C for 30 minutes (Department of Agriculture, Water and the Environment, 2021a). This has been particularly challenging for treatment providers during the northern hemisphere winter, raising further questions on the efficacy of this modality in other European countries during this time of year.

Methyl bromide (MB) fumigation accounts for 29% of offshore biosecurity treatments for BMSB. Whilst methyl bromide is effective in controlling BMSB, the chemical is recognised as an ozone depleting substance, and under the Montreal Protocol, has been gradually phased out as a biosecurity treatment since 2005 (Ormsby, 2018; Park et al., 2020). Research by Baur et al. (2015) also found that approximately 10 to 20% of sea freight arriving in Europe contained unsafe concentrations of methyl bromide. This indicates that unsafe exposure to methyl bromide is a risk to not only fumigators, but those down the transport chain, including inspectors, importers and their customers. The typical ventilation processes used following fumigation may be a contributing factor to gas concentrations remining at an unsafe level (natural or fan forced) have also found to be largely ineffective in lowering gas concentrations (Svedberg and Johanson, 2013). Whilst the toxicity of this fumigant towards BMSB is clearly high, its negative environmental effects ensure that it is not viable long term.

Sulfuryl fluoride (SF) is the most widely employed offshore biosecurity treatment for BMSB, accounting for 45% of treatments conducted. Sulfuryl fluoride is an inert quarantine fumigant that can effectively control a variety of insect species and life stages (Bell, 2006). Furthermore, Sulfuryl fluoride has been found to be generally more toxic towards insect pests than its primary counterpart, methyl bromide (Johnson et al., 2012). However, as a fluorinated gas, sulfuryl fluoride is classified as a greenhouse gas with global warming potential 4780 times higher than carbon dioxide (United States Environmental Protection Agency, 2021; Papadimitriou et al., 2008). In recent years, it has also been discovered that sulfuryl fluoride is accumulating in the atmosphere at a rate six to ten times greater than previously thought (Muhle, 2009). Coupled with sulfuryl fluoride's odourless and colourless characteristics, there have been concerns from the shipping industry and government regarding the safety of residue levels in containers entering Australia. During the 2019-20 BMSB season concerns regarding sulfuryl fluoride residue culminated in DAWE quarantine staff refusing to inspect inbound freight treated with this fumigant (Shipping Australia Limited, 2019). As a result, SF cannot be a standalone replacement for MB due to the substantial occupational health and environmental risks posed. There is also a risk that its high toxicity to humans results in further reluctance from quarantine staff to inspect freight treated with this chemical or result in less extensive inspections to reduce their exposure time.

Ethyl formate is a potential alternative biosecurity treatment for the control of BMSB. This chemical is a low-molecular-weight volatile compound produced by many fruits and vegetables and has an important role as a flavour and aroma component (National Center for Biotechnology Information, 2021). In the last decade, ethyl formate has been successfully deployed as a fumigation treatment for invasive pests in cereals and a variety of fruits such as bananas, grapes and citrus (Krishna et al., 2005; Ryan and De Lima, 2014). The rise of ethyl formate as a fumigant is largely a result of its fast action, strong penetration and degradation

to biogenic substances ethanol and formic acid (Muthu et al., 1984; Ren and Desmarchelier, 2002). Ethyl formate is substantially less toxic to humans than its primary fumigant competitors. Due to of the comparatively low toxicity of ethyl formate, and its food grade status, it can be considered as a significantly safer fumigant from both an occupational safety and environmental standpoint. In Australia, ethyl formate is registered for use as a dried fruit fumigant (Hilton and Banks, 1997); however, the chemical properties of ethyl formate outlined above also make it an ideal fumigant for biosecurity applications.

2.5. Conclusions

This study has found that our wine industry is in a vulnerable position due to the insect potentially hitchhiking on inbound sea freight into Australia, with numerous post-border detections of the insect occurring in the last five years. Financial modelling indicates that if the BMSB were to establish in Australia, it could have a long-term financial impact on the industry in excess of $300 million. The key findings suggest that whilst heat treatment may be the cheapest treatment method, its efficacy is inconsistent. Methyl bromide and sulfuryl fluoride are both relatively expensive and lengthy treatments, that whilst effective, present serious occupational health and environmental concerns. Ethyl formate is proposed as a viable alternative

Chapter 3: Ethyl formate is a biosecurity solution for controlling brown marmorated stink bug (*Halyomorpha halys*)

3.1. Introduction

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), commonly referred to as the brown marmorated stink bug (BMSB) is a polyphagous insect capable of feeding upon over 300 species of plants (Lee et al., 2013a), ranging from fruit and vegetables to native vegetation. The insect's most likely route of entry into new countries/territories/regions is via the shipping of vehicles and other machinery from infested regions (Hoebeke and Carter, 2003).

Currently, the greatest probability for successful BMSB establishment in Australia is from the northern hemisphere (Ormsby, 2018). This is predominantly due to BMSB infesting cargo prior to shipping to overwinter in the northern hemisphere (Ormsby, 2018). Once cargo enters warmer temperatures in the southern hemisphere, it acts as a catalyst to end overwintering and initiate reproductive cycles (Niva and Takeda, 2003). Overwintering BMSB usually enter diapause, a physiological state many insects go through that reduces their metabolism and respiration, which significantly increases their tolerance to chemical treatments (Leskey and Nielsen, 2018; Bell, 1977). Due to the inaccessibility of many areas within shipping containers and machinery, fumigation is the preferred and most effective treatment. Due to the BMSB threat posed to Australia, seasonal treatment measures are implemented from September to April each year, coinciding with the north hemisphere winter (Department of Agriculture, Water and the Environment, 2021a)

Research conducted by Abrams et al. (2020) on adult BMSB found a two-fold increase in fumigation tolerance to sulfuryl fluoride in diapausing insects when compared to non-diapausing. To achieve high mortality, insects must be exposed to a sufficient concentration of a fumigant and/or exposure period, to enable penetration through the commodity in which the insect is harbouring without decay prior to the eradication of all life stages (Bond, 1975).

Sulfuryl fluoride and methyl bromide are currently used throughout the world for the fumigation of shipping containers and machinery suspected of harbouring BMSB (Department of Agriculture, Water and the Environment, 2021a). Methyl bromide is being phased out as part of the Montreal Protocol, due to it being identified as an ozone depleting substance (United Nations Environment Programme, 2006).

As reported by Derrick et al. (1990), methyl bromide is also reactive with rubber, resulting in perishing of the product, thus making it unsuitable for the treatment of vehicles and machinery because of possible damage to tyres and rubber sealants. Unlike methyl bromide, sulfuryl fluoride is an entirely synthetic and inert compound, making it highly unreactive (Derrick, 1990) and, as a result, sulfuryl fluoride is the internationally preferred fumigant for many vehicle manufacturers (Ormsby, 2018). Research by Abrams et al. (2020) found sulphuryl fluoride to be capable of achieving Probit 9 mortality for adult diapausing BMSB at a concentration by time product (Ct) of 585.1 mg h/L. Probit 9 refers to the International Plant Protection Convention's efficacy standard of 99.9968% mortality for phytosanitary treatments, that is, representing one surviving insect out of over 93,000 (International Plant Protection Convention, 2015). Bioassays on other insect pests have found sulfuryl fluoride to be less effective against immature life stages (Ormsby, 2018). There have also been concerns surrounding sulfuryl fluoride's environmental impact, notably its classification as a greenhouse gas (Muhle et al., 2009) and acute fluoride toxicity (National Center for Biotechnology Information, 2021). As a result, there is a need for an alternative fumigant which is both effective for the treatment of BMSB and environmentally friendly.

Ethyl formate is a possible alternative fumigant for the treatment of BMSB and other insect pests. It is a low-molecular-weight volatile compound produced by many fruits and vegetables. It has an important role as a food grade flavour and aroma component (Vu and

Ren, 2004) and it has been used as a fumigant for the treatment of dried fruit (Hilton and Banks, 1997) and stored grain (Muthu et al., 1984).

The rise of ethyl formate as a fumigant is largely a result of its fast action, strong penetration and quick breakdown to the biogenic substances ethanol and formic acid (Muthu et al., 1984; Ren and Desmarchelier, 2002). Relevant to this study, research conducted by Kawagoe et al. (2017) on non-diapausing BMSB with a two-hour exposure period at 10°C, found that ethyl formate was effective in achieving LD_{99} (lethal dose equal to 99% mortality) at 10.26 mg/L, and Probit 9 mortality at 16.5 mg/L.

The objective of this research is to evaluate, through both controlled laboratory and commercial scale trials, the efficacy of ethyl formate to control adult BMSB at the temperatures and dormancy states encountered during seasonal biosecurity treatments.

3.2. Materials and Methods

3.2.1. Test insects

For laboratory ethyl formate fumigation bioassays, adult BMSB were field collected in Susong County, Anhui, China (30°08' N 116°05' E) from the 12th to the 13th of May, 2020. The insects were first held in an insect culture room at 26±1 °C and 60-65% RH and 11:13 Light:Dark [L:D]) h, and fed with broad bean (*Vicia faba* L.). The insects were placed into insect cages (35 x 35 x 35 cm), each holding approximately 500-600 adult BMSB (Figure 3-1). The cages were transferred to the Chinese Academy of Inspection and Quarantine (CAIQ) Beijing laboratory.

BMSB were induced to diapause using a modified method as reported in Abrams (2020). After first molt, cohorts of insects in “rearing” enclosures were transferred to an incubator set to 20 °C, 65% RH and 11:13 [L:D] h. Following emergence as adults in approximately 38

days, insect samples were held for an additional week at 20 °C. After a week the diapause enclosures were transferred to a second incubator set to 15 °C, 65% RH and 11:13 [L:D] h. Feeding typically stopped 1–2 weeks prior to the insects being prepared for fumigation; i.e. commencing diapause.

Figure 3-1. Insect collection in the field (top images) and experimental cages (bottom images).

3.2.2. Ethyl formate calculations

Liquid ethyl formate (>99.0% purity) sourced from Sigma Aldrich, Sydney, Australia was used for all fumigation protocols. The dosages and required volumes of liquid ethyl formate for the fumigant concentrations were calculated from Equation 3-1:

$$\mathbf{V_f = \frac{C \times V}{P \times D}}$$ ……...Equation 3-1

Where: V_f is the experimental volume to be applied (mL),

V is the volume of the fumigation container (L),

P is purity of liquid EF (%), C is the intended concentration of fumigant (mg/L),

D is density of liquid EF.

The concentration of ethyl formate (Figure 3-2) during experimental fumigations was analysed via gas chromatography (Agilent 6890N Gas chromatograph) equipped with a Flame Ionization Detector (GC-FID). Detection conditions were: Injection port temperature 200℃; Packed Chromatographic column Propark Q (80-100 mesh); Column temperature 120℃; Carrier gas: H_2; Column flow rate: 0.4 mL/min.

The ethyl formate standard gas was prepared using liquid EF (>99% purity, balanced with ethanol) at concentrations of 20, 40, 60, 80, 100 mg/L. After three repeat injections, the mean peak area was fitted linearly with the standard gas concentration at each point, creating an equation for the EF standard curve (Figure 3-2)

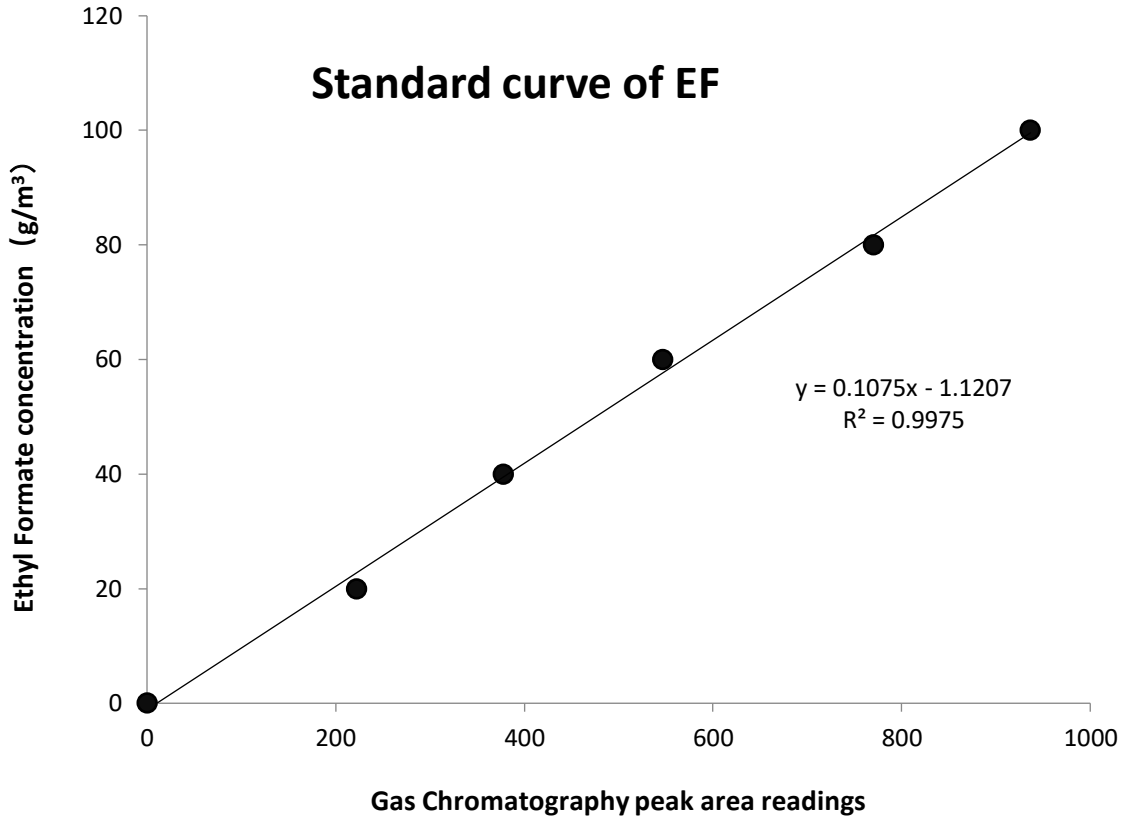

Figure 3-2. Gas Chromatography-Flame Ionisation Detector standard curve of ethyl formate.

The concentration of ethyl formate was monitored at timed intervals (10, 60, 120, and 180 minutes) over the exposure period (3 hours) via the extraction of gas samples with a 100 µL gas-tight syringe, and was used to calculate the product ***C***t = concentration time with Equation 3-2).

$$Ct = \sum(C_i + C_{i+1})\,(t_{i+1} + t_i)/2 \quad \text{.....Equation 3-2}$$

Where: **C** is ethyl formate concentration (mg/L),

t is time of exposure (hours),

i is the order of measurement,

C*t* is concentration × time product (mg h/L).

3.2.3. Laboratory fumigation trials

The conditioned fumigation chambers containing adult brown marmorated stink bugs were sealed and placed in temperature-controlled cabinets at 10 °C or 25 °C and 62.5-65% RH. A 7 cm diameter filter paper (Whatman No. 1) was inserted into the glass lid to provide a liquid evaporation surface for the injected ethyl formate.

The fumigation trials were conducted at 10 °C and 25 °C and 60% RH in 2.2 L fumigation chambers fitted with a gas sampling port. Between 100 and 110 diapausing and non-diapausing adult insects were placed in the unsealed chambers and left overnight at 10 °C or 25 °C and 62.5-65% RH prior to the fumigation treatment the next morning. The diapausing insects were taken straight from the culture cabinet to the treatment chambers.

According to previous studies, the target concentrations of ethyl formate used for the 3-hour exposure treatments were 2, 4, 6, 8, 10, 12, and 14 mg/L. After removal of the same volume of air as the injected vaporised fumigant, the fumigant was injected into the chamber and stirred with two computer cooling fans (AC Infinity Multifan S1 Quiet 8cm USB Fan, Japan. Perth, Australia). The dosage (calculated by Equation 3-1) was injected into the chambers using a liquid syringe (SGE, Melbourne, Australia; Microliter syringe 1025 TLL 25 ml). The control insects were maintained in a sealed chamber without the application of ethyl formate until completion of exposure. Each fumigation bioassay treated a minimum of 1500 insects, with a minimum of 300 insects used as untreated controls. Each treatment involved three replicates of each ethyl formate concentration used, with three control replicates.

The concentration of ethyl formate was monitored during the fumigation period. Gas samples (80 µL) were taken from the fumigation chambers with a 100 µL gas tight

syringe 2-5 minutes following the injection of fumigant, and prior to opening the chambers; and were then injected into the GC-FID. Prepared ethyl formate gas standards were used to determine EF concentration in the chambers.

At the end of the fumigation period of 3 hours, both treated and control chambers were opened for 1 hour of ventilation in a fume hood at 25 °C and 60% RH. Bioassay samples were retrieved at the end of the fumigation period, the live and dead adult insects were counted and transferred to an incubator set at 25 °C and 65% RH for four days to ensure diapause ended and then the live and dead adult insects were counted to determine end-point mortality.

3.2.4. Commercial Fumigation Trials

Fumigation was carried out using a 20-foot refrigerated shipping container. Prior to fumigation, a gas tightness test was conducted by connecting an air pump to gas sample lines inserted into the container. A pressure detection probe was also inserted into the container. Air was pumped into the container until the pressure reading exceeded 40 pascals (pa). The time for the container's internal pressure to decrease from 40 to 20 pa was recorded. The time recorded was greater than 8 seconds, and thus the container was recognised as having a suitable gas tightness for fumigation (De Lima et al., 1994).

Prior to fumigation, seven gas sampling lines were arranged at the left rear bottom, right rear top, central top, central middle, central bottom, left front top and front bottom right. The 2 temperature and humidity monitors (Tracksense-pro, Ellab, Denmark) were located at rear top and front bottom in the shipping container (Figures 3-3 and 3-4). Finally, 21 insect cages were placed as shown in Figure 3-4.

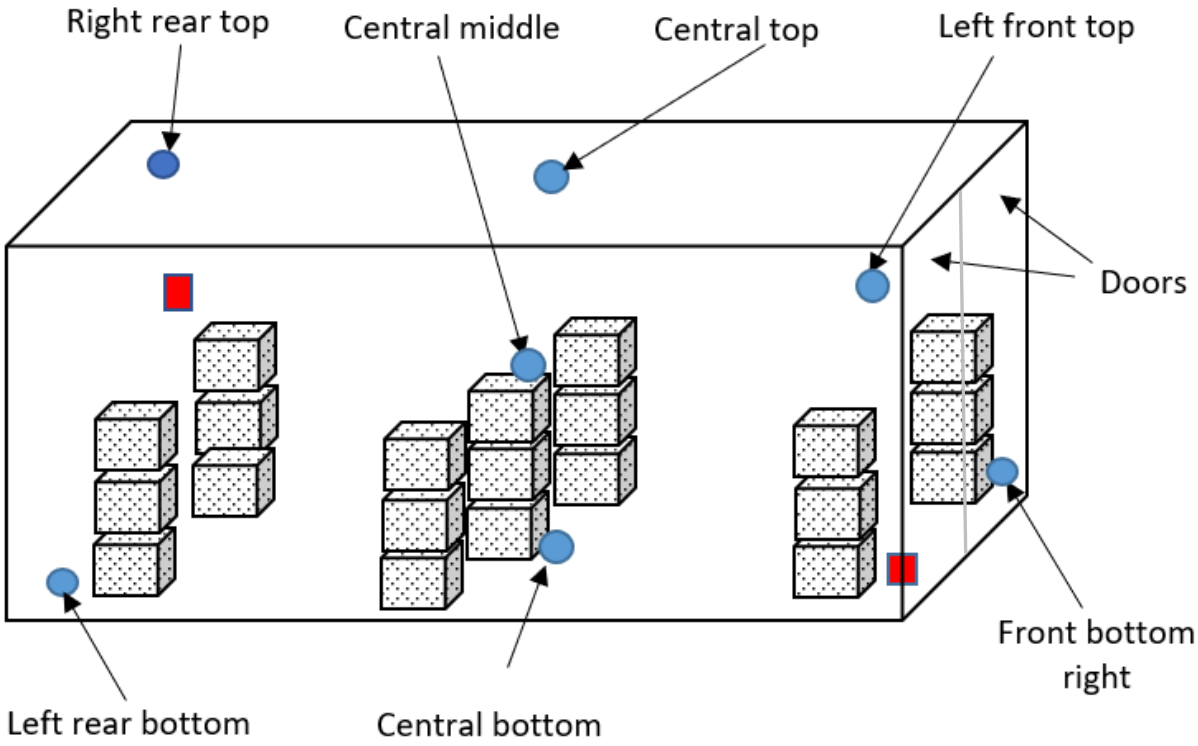

Figure 3-3. Placement of fumigant sampling ports (●), temperature and RH sensors (■), and insect cages (▣) within the fumigated container.

Figure 3-4. Insect cages within shipping container used for commercial fumigation trials.

Three doses of ethyl formate were used based on the findings of the laboratory work and the research conducted by Coetzee et al., (2021): 10, 40 and 60 mg/L. A boiler was used to vaporise the liquid ethyl formate and purged with cylinderised 99.5% purity nitrogen into the container. A 40 cm domestic fan was placed inside the container for stirring ethyl formate gas to ensure even distribution within the container.

During the 3-hour holding period, ethyl formate gas samples were collected with an electric gas pump (FASCO, Model: CAPEX L2, Charles Austen Pumps Ltd, UK) drawing gas at 10 min, 30 min, 1, 2 and 3 h after application from each gas sampling line into 1-L Tedlar® gas bags.

3.2.5. Mortality assessments

3.2.5.1. Laboratory Fumigation Trials

Mortality was assessed by counting dead diapausing and non-diapausing adult insects 48 hours post exposure (recovery time); and compared with the control held at the same exposure temperature and relative humidity. Mortality was determined by a lack of coordinated movement after probing the insects with a soft haired paint brush.

The toxicity of ethyl formate to the diapausing and non-diapausing BMSB was calculated from the level of mortality in more than 6,500 adult insects exposed to different concentrations of EF under different treatment temperatures. Normally, mortality would be corrected with Abbott's (1925) formula. However, this bioassay, more than 1,200 untreated control insects were used and was no mortality in any of the control bioassays was observed within 3 hours short holding period.

3.2.5.2. Commercial Scale Trials

Mortality was assessed by counting dead and live non-diapausing adult insects. The insect cages were removed from the container following exposure with mortality assessments being conducted within a controlled laboratory setting. To ensure the insects were dead and not narcotised, mortality assessments were conducted at 24, 48, 72 and 96 hours. Mortality was also determined via the use of a soft haired paintbrush identical to the laboratory fumigation trials.

3.2.6. Data Analyses

Bioassays with ethyl formate that achieved 100% mortality were excluded from the probit analyses, in line with the methodology used in Burgess et al. (2020).

SPSS software (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) was used to fit the Probit model to the data (Finney, 1947). Non-overlapping 95% confidence

intervals (CIs) between different treatments i.e., diapause vs non-diapause at two different temperatures of 10 °C and 25 °C, were assessed as significantly different at $P < 0.05$. The concentration of ethyl formate was transformed to base 10 logarithmic ($\log_{10}$ concentration). Analyses outputs include the intercept, slope, and SE of the slope; the fitted individual equations at the LD_{50} values along with their CIs; and the chi-square goodness-of-fit test statistics and associated p-values. Further LD_{50} and LD_{99} values are also given with the CIs (both the log-transformed values and the back transformed values on the original scale are given).

Probit 9 ($LD_{99.9968}$) mortality was calculated differently using Microsoft Excel software (Microsoft Corporation, 2018.) with a methodology described by Finney (1971) and Currell (2015) where probit values are calculated from percent mortality as 5 plus the inverse of the standard of the normal distribution (5+NORM.S.INV). Probit 9 was calculated by extrapolation using the equation for the regression line and finding the anti-log of the predicted dosage.

The natural response rate was calculated within the model fit in Statistical Product and Service Solutions (SPSS) software (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) using the control mortality. The natural response rate is the probability that an insect dies without being exposed to any external stressor such as chemical.

3.3. Results

3.3.1. Laboratory Bioassays

The results obtained from the laboratory ethyl formate fumigation of adult BMSBs at two temperature conditions and dormancy states indicate that complete insect control was achieved across all conditions at concentrations greater than 12 mg/L (Tables 3-1 to 3- 4).

The results also show that higher concentrations of ethyl formate were required to achieve high levels of mortality in diapausing insects compared to non-diapausing insects (Tables 3-1 to 3-4). This was reflected in the calculated LD_{50} (lethal dose required to achieve 50% mortality) where 10 °C diapause required the highest calculated concentration (6.412 mg/L), followed by 25 °C diapause (5.508 mg/L), 10 °C non-diapause (4.634 mg/L) and 25 °C non-diapause (4.036 mg/L) (Table 3-5). The confidence intervals for LD_{50} (Table 3-5) showed that 10 °C and 25 °C diapause did not overlap, and as such were significantly different to each other or to 10 °C and 25 °C non-diapause (which also did not overlap). Treatment conditions 10 °C non-diapause and 25 °C non-diapause did have overlapping confidence intervals and were not significantly different from each other.

The calculated LD_{99} (lethal dose required to achieve 99% mortality) for each treatment condition followed the same pattern observed with LD_{50}. Diapausing insects at 10 °C were the most difficult to kill (12.106 mg/L), followed by 25 °C diapause (10.471 mg/L), 10 °C non-diapause (8.872 mg/L) and 25 °C non-diapause (8.433 mg/L) (Table 3-6). The confidence intervals calculated for LD_{99} (Table 3-6) show an overlap between results from 10 °C and 25 °C, indicating that they were not significantly different. Overlapping confidence intervals were also found between 25 °C diapause and the two non-diapause conditions (10 °C and 25 °C), indicating they were not significantly different from each other. However, 10 °C diapause was significantly different from treatments at 10 °C and 25 °C non-diapause.

The regression curves (Figure 3-5) calculated from the results demonstrate the mortality relationship between each condition, and highlight the increased dosage demanded in treating diapausing insects.

The calculated Probit 9 (Table 3-5 and Figure 3-5) for each treatment condition is equivalent to 99.9968% mortality. The calculated Probit 9 values were 23.51 mg/L for Diapause 10 °C,

19.88 mg/L for Diapause 25 °C, 15.98 mg/L for Non-diapause 10 °C, and 14.49 mg/L for Non-Diapause 25 °C. Due to the absence of control mortality, Abbott's formula was not required. The measured high fumigant concentration for each condition was used for analysis.

Goodness of fit analysis (Table 3-7) show that Diapause 10 °C and Non-diapause 10 °C had statistically insignificant p-values, and as such, that the Probit model adequately fitted the data. On the other hand, goodness of fit analysis showed that Diapause 25 °C and Non-Diapause 25 °C had statistically significant p-values, and as such the Probit model was not a good fit.

Table 3-1. Mortality of brown marmorated stink bug at 10 °C for 3 hours exposure to ethyl formate: Diapause condition.

EF concentration (mg/L)			**Number of Insects Tested**	
Low	**High**	**Mean**	**Dead**	**Total**
2.15	2.57	2.36	2	103
2.21	2.59	2.40	4	100
2.26	2.79	2.53	1	102
4.90	5.79	5.34	39	103
4.94	6.06	5.50	37	99
5.02	5.75	5.39	40	100
6.02	8.20	7.11	81	101
6.59	8.73	7.66	83	101
6.85	8.14	7.49	85	98
8.06	9.98	9.02	98	100
8.17	10.11	9.14	98	103
8.35	10.58	9.47	99	102
9.53	12.78	11.16	100	101
9.58	11.22	10.40	102	102
9.49	12.51	11.00	99	100
10.95	13.13	12.04	99	99
12.04	14.11	13.08	103	103
12.21	13.96	13.09	101	101
Total			1271	1818
Control			0	102
Control			0	98
Control			0	101
Total			0	301

Table 3-2. Mortality of brown marmorated stink bug at 10 °C for 3 hours exposure to ethyl formate: Non-diapause condition.

EF concentration (mg/L)			Number of Insects Tested	
Low	**High**	**Mean**	**Dead**	**Total**
1.76	2.34	2.05	1	100
1.80	2.35	2.08	1	102
1.81	2.54	2.18	1	103
4.91	5.26	5.09	72	103
4.93	5.51	5.22	71	101
4.93	5.23	5.08	68	102
6.50	7.45	6.98	95	100
6.57	7.93	7.25	101	104
5.68	8.33	7.01	99	101
8.10	11.10	9.60	102	103
8.23	10.20	9.22	102	102
8.47	11.93	10.20	110	110
12.13	14.08	13.11	104	104
12.53	14.51	13.52	100	100
13.43	15.11	14.27	103	103
Total			1130	1538
Control			0	100
Control			0	101
Control			0	103
Total			0	304

Table 3-3. Mortality of brown marmorated stink bug at 25 °C for 3 hours exposure to ethyl formate: Diapause condition.

EF concentration (mg/L)			Number of Insects Tested	
Low	**High**	**Mean**	**Dead**	**Total**
2.08	2.33	2.20	2	98
2.09	2.29	2.19	3	103
2.09	2.31	2.20	2	103
3.14	4.18	3.66	25	103
3.21	4.20	3.71	19	102
3.23	4.20	3.72	21	103
4.63	5.87	5.25	56	101
5.08	6.11	5.60	58	102
5.15	6.52	5.84	60	101
6.60	7.01	6.81	89	102
6.00	7.33	6.67	88	99
6.14	7.03	6.59	86	100
8.67	9.06	8.86	99	100
8.21	10.11	9.16	102	103
9.05	10.25	9.65	101	101
11.83	13.66	12.75	102	102
12.06	13.40	12.73	100	100
11.94	13.02	12.48	103	103
Total			1116	1826
Control			0	102
Control			0	100
Control			0	103
Total			0	305

Table 3-4. Mortality of brown marmorated stink bug at 25 °C for 3 hours exposure to ethyl formate: Non-diapause condition.

EF concentration (mg/L)			Number of Insects Tested	
Low	**High**	**Mean**	**Dead**	**Total**
1.65	2.10	1.88	2	98
1.70	2.23	1.97	1	98
1.74	2.30	2.02	2	102
3.77	4.98	4.38	62	101
3.80	4.12	3.96	65	100
3.86	4.23	4.05	64	101
4.63	5.87	5.25	90	100
5.07	6.73	5.90	93	98
5.27	7.27	6.27	94	98
8.21	9.37	8.79	98	99
8.36	9.75	9.06	102	102
8.81	9.92	9.37	103	103
11.60	13.18	12.39	100	100
11.70	13.46	12.58	103	103
12.10	13.93	13.02	101	101
Total			1080	1504
Control			0	101
Control			0	102
Control			0	98
Total			0	301

Table 3-5. Calculated LD_{50} mortality of brown marmorated stink bug for 3 hours exposure to ethyl formate: All conditions.

Insect Condition	**Treatment Temp**	**Pooled Number of BMSB**	**3h Log10 LD_{50} Value (95% CI)**	**3h LD_{50} Value (95% CI)**
Diapause	10°C	1320	.807 (.791 - .820)[a]	6.412 (6.180 - 6.607)[a]
Diapause	25°C	1714	.741 (.718 - .761)[b]	5.508 (5.224 -5.768)[b]
Non-diapause	10°C	1296	.666 (.636 - .689)[c]	4.634 (4.325 - 4.887)[c]
Non-diapause	25°C	1725	.606 (.551 - .645)[c]	4.036 (3.556 - 4.416)[c]

LD_{50} values followed by different letters are significantly different from each other due to the 95% confidence intervals overlapping.

Table 3-6. Calculated LD_{99} mortality of brown marmorated stink bug for 3 hours exposure to ethyl formate: All conditions.

Insect Condition	Treatment Temp	Pooled Number of BMSB	3h Log10 LD_{99} Value (95% CI)	3h LD_{99} Value (95% CI)
Diapause	10°C	1320	1.083 (1.058 - 1.114)[a]	12.106 (11.429 - 13.002)[a]
Diapause	25°C	1714	1.020 (.978 - 1.081)[a,b]	10.471 (9.506 -12.050)[a,b]
Non-diapause	10°C	1296	.948 (.914 - .997)[b]	8.872 (8.204 - 9.931)[b]
Non-diapause	25°C	1725	.926 (.864 - 1.030)[b]	8.433 (7.311 - 10.715)[b]

LD_{99} values followed by different letters are significantly different from each other due to the 95% confidence intervals overlapping.

Table 3-7: Probit analysis parameter estimates for regression curves: All conditions

Treatment Condition	Parameter	Estimate	Std. Error	Z	Sig.	95% CI Lower Bound	95% CI Upper Bound
Diapause 10°C	High Conc	8.421	.506	16.631	.000	7.428	9.413
	Intercept	-6.794	.445	-15.284	.000	-7.239	-6.350
Diapause 25°C	High Conc	7.265	.504	14.427	.000	6.278	8.252
	Intercept	-4.400	.355	-12.401	.000	-4.755	-4.045
Non-Diapause 10°C	High Conc	8.261	.843	9.797	.000	6.608	9.913
	Intercept	-5.504	.649	-8.479	.000	-6.154	-4.855
Non-Diapause 25°C	High Conc	8.330	.486	17.140	.000	7.377	9.282
	Intercept	-6.172	.384	-16.069	.000	-6.556	-5.788

PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using Log_{10}).

Table 3-8. Goodness of fit analysis for all fumigation treatment conditions

Insect Condition	Treatment Temperature	Analysis	Statistical Model	Chi-Square	df	Sig.
		PROBIT	Pearson Goodness-of-Fit Test			
Diapause	10°C			11.372	11	.413a
Diapause	25°C			24.721	11	.010a
Non-Diapause	10°C			13.228	7	.067a
Non-Diapause	25°C			21.745	7	.003a

a. Since the significance level is less than .150, a heterogeneity factor is used for the calculation of confidence limits

Table 3-9. Regression equations for mortality of brown marmorated stink bug for 3 hours exposure to ethyl formate: All conditions.

Insect Condition	Treatment Temperature	3h Log10 LD_{50} Value	Regression Equation
Diapause	10°C	.807	Y=-6.794+8.421*log10_ Concentration (mg/L) of ethyl formate
Diapause	25°C	.741	Y=-4.40 +7.265*log10_ Concentration (mg/L) of ethyl formate
Non-Diapause	10°C	.666	Y=-5.504 +8.261 *Log10 Concentration (mg/L) of ethyl formate
Non-Diapause	25°C	.606	Y=-6.172+8.330* Log10_ Concentration (mg/L) of ethyl formate

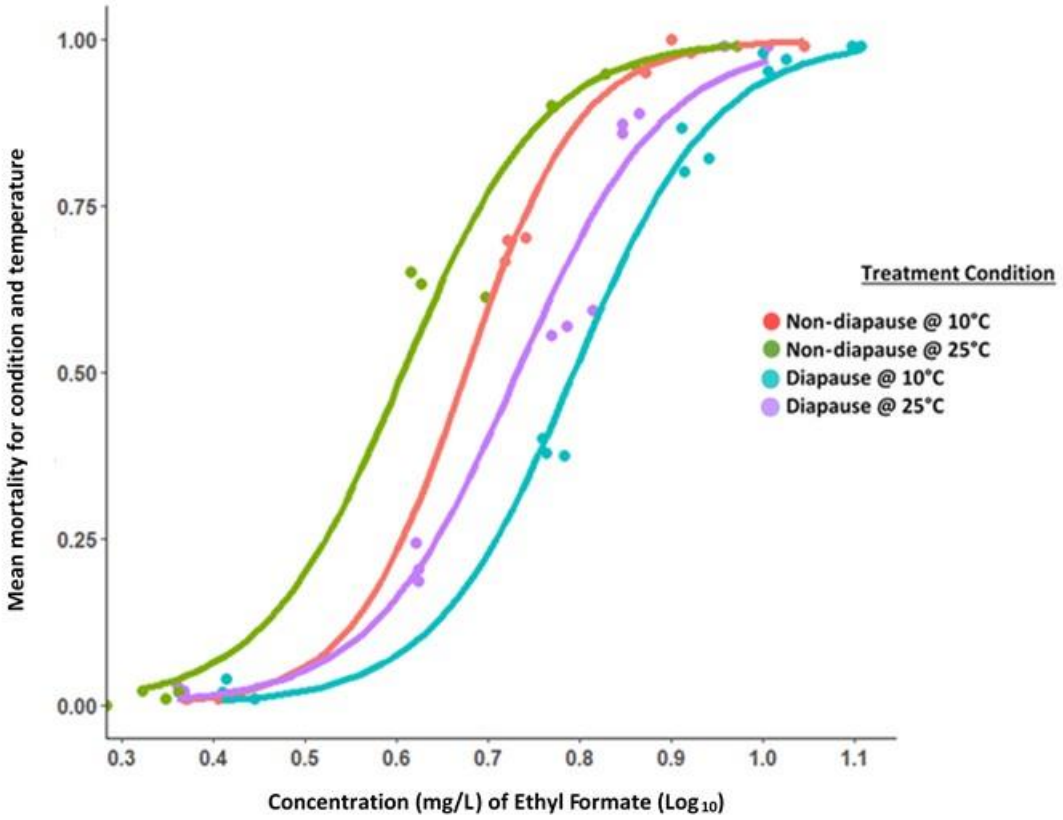

Figure 3-5. Log_{10} mortality of brown marmorated stink bug for 3 hours exposure to ethyl formate: All conditions.

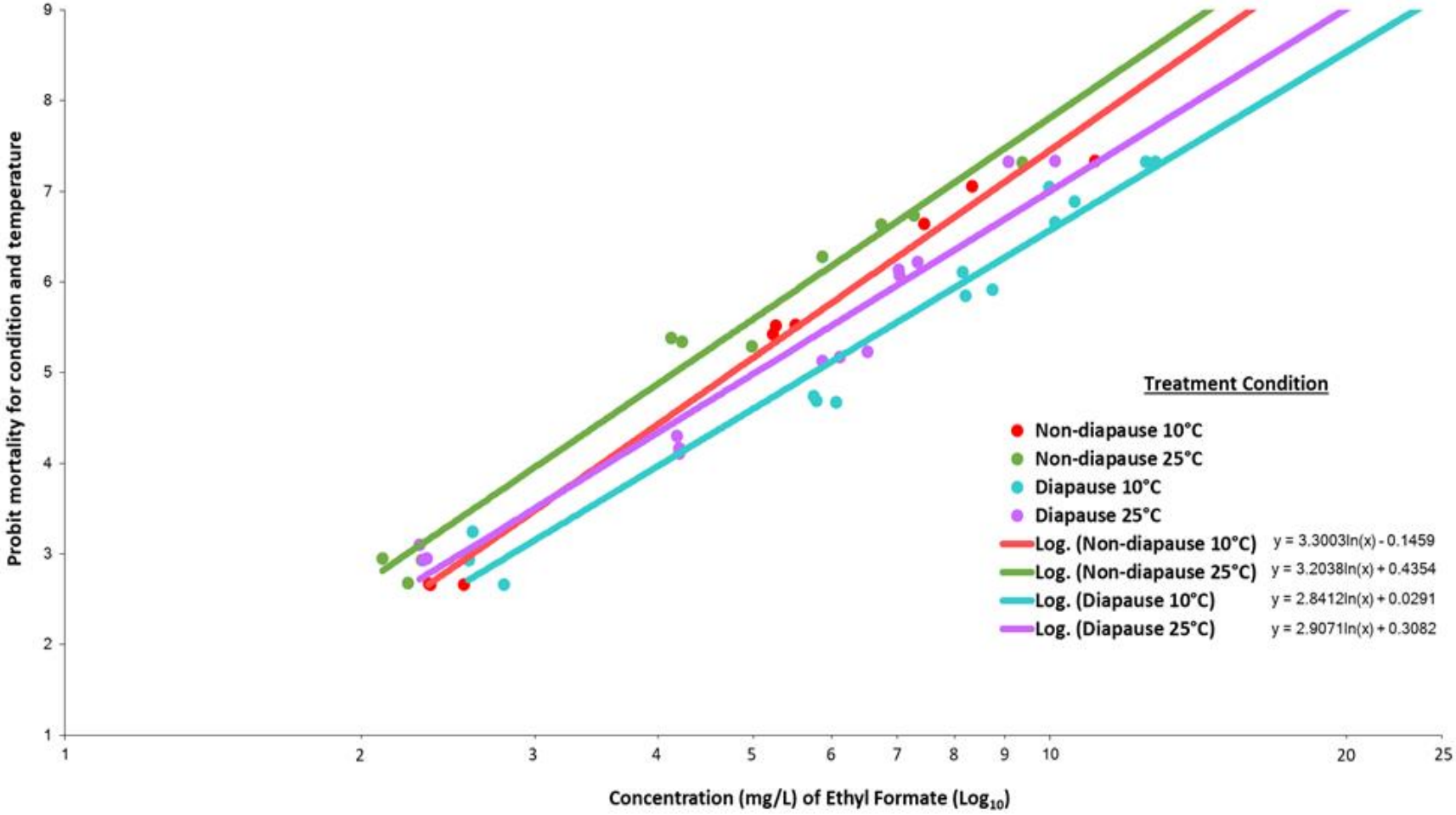

Figure 3-6. Probit mortality of brown marmorated stink bug for 3 hours exposure to ethyl formate: All conditions.

3.3.2. Commercial Fumigation Trials

The three commercial scale fumigation trials found that concentrations equal or above 10 mg/L were sufficient to achieve complete insect control in non-diapausing adult insects (Table 3-9). In this study, the three ethyl formate concentrations used (10, 40 and 60 mg/L) achieved between 193.86 and 24.5 **C***t* (Tables 3-9, 10, 11, 12). In all three treatments, the complete mortality was achieved within one day (Table 3-12).

Table 3-10. Treated insect mortality at three time points (1, 2, 4 days) following exposure: All trials (>10 mg/L).

Insect Mortality Post Fumigation (>10 mg/L)

	Day 1		Day 2		Day 4	
	Live	Dead	Live	Dead	Live	Dead
Trial 1 (60 mg/L)	0	11230	0	11230	0	11230
Trial 2 (40 mg/L)	0	13500	0	13500	0	13500
Trial 3 (10 mg/L)	0	12650	0	12650	0	12650

Table 3-11. Trial 1 (60 mg/L) ethyl formate fumigation gas concentrations at time intervals and calculated Concentration by Time product.

Trial 1 Fumigation (60 mg/L)						
Temperature	18-19.5 ℃					
Relative humidity	62-66%					
Gas tightness (Half-life)	8 seconds					
	Sampled EF concentrations（mg/L）					
Sample Ports	10min	30min	1h	2h	3h	**C***t*
#1	63.62	57.26	60.30	56.47	58.65	175.55
#2	67.14	50.26	57.09	74.42	69.42	193.86
#3	57.13	53.63	57.65	65.41	53.22	176.35
#4	58.33	60.85	49.49	59.81	56.32	170.09
#5	48.20	57.67	57.75	64.41	53.33	175.27
#6	50.03	54.19	56.03	70.19	52.75	178.19
#7	58.83	56.38	62.43	65.14	56.61	183.17

Table 3-12. Trial 2 (40 mg/L) ethyl formate fumigation gas concentrations at time intervals and calculated Concentration by Time product.

Trial 2 Fumigation (40 mg/L)						
Temperature	18-19.5 ℃					
Relative humidity	62-68%					
Gas tightness (Half-life)	8 seconds					
	Sampled EF concentrations（mg/L）					
Sample Ports	10min	30min	1h	2h	3h	**C***t*
#1	37.60	35.99	43.13	37.03	31.84	112.69
#2	46.39	41.02	45.81	45.95	18.86	121.85
#3	27.29	48.78	42.12	30.25	32.29	109.20
#4	36.39	51.43	36.28	45.10	47.24	130.80
#5	40.83	48.59	44.84	45.46	32.46	129.82
#6	41.00	62.24	35.26	41.63	32.55	125.72
#7	45.60	40.27	37.39	41.66	28.04	115.26

Table 3-13. Trial 3 (10 mg/L) ethyl formate fumigation gas concentrations at time intervals and calculated Concentration by Time product.

Trial 3 Fumigation (10 mg/L)						
Temperature	18-19.5 ℃					
Relative humidity	62-68%					
Gas tightness (Half-life)	8 seconds					
	Sampled EF concentrations（mg/L）					
Sample Ports	10min	30min	1h	2h	3h	**C***t*
#1	11.18	9.88	14.03	9.35	6.03	30.62
#2	13.61	13.70	11.77	10.61	5.16	32.27
#3	8.35	9.02	8.73	11.79	5.81	27.84
#4	11.33	9.51	13.64	13.79	4.45	33.83
#5	10.90	11.21	13.77	9.77	5.66	31.26
#6	10.18	10.20	11.10	5.67	5.72	24.50
#7	11.74	15.79	17.11	2.49	6.17	29.20

3.4. Discussion

Ethyl formate was shown to be effective at applied concentrations above 12 mg/L for the control of adult BMSB irrespective of dormancy state and temperature conditions tested. The laboratory bioassay found that diapausing insects, as expected, were the most difficult to kill, with the lower temperature condition (10 °C) requiring a higher ethyl formate concentration than the 25 °C temperature condition to achieve the desired mortality level (LD_{50}, LD_{99} and Probit 9). The results from the laboratory bioassays are follow existing literature on the

fumigation of insects, where there is an inverse relationship between temperature and the fumigant concentration required to achieve mortality (Bond and Monro, 1984).

As respiration is the fumigant's primary pathway of entry into the insect, the positive relationship between increases in temperature and rate of respiration explain the lower required fumigant concentration (Sun, 1946). This aligns with the Australian Government regulations, which do not permit quarantine and pre-shipment fumigations to be conducted below 10 °C (Department of Agriculture, Water and the Environment, 2018).

The laboratory bioassay results followed those from a similar study by Kawagoe et al. (2017). Despite the exposure time for Kawagoe et al. (2017) being only 2 hours in comparison to the 3 hours in this work, the results were comparable. In these laboratory bioassays, 10 °C non-diapause treatments had an LD_{99} of 8.87 mg/L and a Probit 9 of 15.98 mg/L; whilst Kawagoe et al. (2017) had a Probit 9 of 16.5 mg/L for a 2-hour exposure, and a Probit 9 of 10.5 mg/L for 4 hours. Both this study and that of Kawagoe et al. (2017) contrast with research evaluating the efficacy of ethyl formate in treating grain insects (Coetzee et al., 2021), where the concentration used to achieve LD_{99} was 90 mg/L, approximately 9 times greater, which indicates that BMSB is more susceptible to EF than *Lasioderma serricorne* (F.), *Sitophilus oryzae* (L.), *Trogoderma variabile* (Ballion), and *Rhyzopertha dominica* (F.). The Australian Government has approved ethyl formate for minor use as a biosecurity treatment for Barrow Island at a **C***t* product 9 times higher than what was required to achieve complete BMSB control in this study (Australian Pesticides and Veterinary Medicine Authority, 2021).

Work described in Abrams et al. (2020) using sulfuryl fluoride found that diapausing BMSB required a gas concentration 2.1 times higher compared to non-diapausing insects at 10 °C to achieve Probit 9. In comparison, this study found diapausing BMSB at 10 °C required a gas concentration (EF) 1.3 times greater than non-diapausing insects to achieve Probit 9. As a

result, it could be assumed that ethyl formate is approximately twice as effective as sulfuryl fluoride at controlling diapausing BMSB.

The commercial scale trials using a 20-foot shipping container validated the laboratory trials and showed that concentrations above 10 mg/L were sufficient to achieve 100% adult insect mortality within a day in each of the three treatments. These results, and the large number of insects tested (n = 37,380) enhance the findings from the laboratory bioassays. While non-diapausing adult insects were used in the commercial trials, it is possible to identify a required ethyl formate concentration to achieve LD_{99} for diapausing insects based on the laboratory scale trials. A control condition was not used due availability of only one 20-foot container and limited equipment; and because laboratory trials indicated that the short exposure time (3 hours) did not result in any control mortality.

This research did not include any commodities in the containers which removed the potential for sorption which would impact on the required rate of fumigant introduced to the container. While this does not directly reflect commercial trade, it does provide strong evidence that ethyl formate is a suitable fumigant to kill diapausing and non-diapausing BMSB. Future commercial scale studies should include a range of commodities and products (vehicles, machinery) to evaluate sorption effects and conduct the trials across a broader range of ambient temperatures. However, if treated at the current registered ethyl formate rate of 90 mg/L, even with sorption these results suggest that 100% mortality would be achieved.

3.5. Conclusions

This study found that ethyl formate is effective in controlling adult BMSB irrespective of dormancy state or temperature (10°C or 25°C), and the fumigant can be used effectively in concentrations 9 times lower than what has been seen in previous studies. The demonstration of achieving LD_{99} or Probit 9 at low temperatures, i.e. 10 °C, is also an important finding for

this research as low temperatures can severely limit the efficacy of fumigation. The concentrations and time of exposure utilised in this study, which achieved 100% mortality were 9 times lower than that currently approved by Australian regulatory authorities and took half the time.

The results clearly demonstrate that ethyl formate is a safe and effective fumigant that represents a strong alternative to what is currently used in the supply chain.

Chapter 4: Efficacy of liquid ethyl formate for controlling stored grain insect pests

4.1 Introduction

Grain is a major component of the Australian agricultural industry, with exports contributing an average of $10.3 billion in revenue to the nation's economy annually (Brown, 2022). Export markets impose strict phytosanitary regulations in order to ensure that product integrity and the economic value of each commodity remain positively correlated (Calhoun, 2015). These phytosanitary regulations are important given that insect infestation can significantly affect grain integrity (Emery, 2017). A principal stage for the detection and treatment of insect infestations is during post-harvest storage.

Fumigation is the preferred treatment modality for post-harvest stored grain products as it is used firstly to protect grain from common endemic insect pests, and secondly for the eradication of exotic insect pests (Emery, 2017). Fumigation treatments utilise chemicals with sufficient toxicity to eradicate the target pest species. These chemicals are pressurised, and temperature controlled before distribution as a gas (Bond, 2007). The vast majority of chemicals are, however, not suitable for application as a fumigant due to their negative environmental, occupational health, and residual effects (Banks, 1990). In recent years, proven fumigants such as methyl bromide have been banned due to their ozone-depleting properties (United Nations Environment Programme, 2006). Phosphine is only available fumigant accepted by world trade, but has fallen out of favour due to insect resistance, resulting from poor fumigation practices, and the length of time required to reach end-point mortality (Xin et al., 2008). This has posed a unique challenge to fumigation chemists throughout the world, leading them to investigate ethyl formate as a possible alternative (Ren and Mahon, 2006).

Over the last decade, ethyl formate has been successfully deployed as a fumigation treatment for invasive pests in cereals and a variety of fruits such as bananas (Krishna et al., 2005), grapes and citrus (Ryan and De Lima, 2014). The rise of ethyl formate as a fumigant is

largely a result of its fast action, strong penetration and degradation to the biogenic substances ethanol and formic acid (Muthu et al., 1984; Ren and Desmarchelier, 2002). The threshold limit value for ethyl formate is 100 ppm, compared to 3 ppm and 0.3 ppm for methyl bromide and phosphine respectively. As such, ethyl formate is 33 and 333 times less toxic to humans respectively than these primary fumigant competitors (Agarwal et al., 2015). Due to of the comparatively low toxicity of ethyl formate, it can be considered as a significantly safer fumigant from both an occupational safety and environmental standpoint.

In Australia, ethyl formate is registered for use as a dried fruit fumigant (Hilton and Banks, 1997; Agarwal et al., 2015); however, the chemical properties of ethyl formate outlined above make it an ideal fumigant for grain and other durable commodities. Research conducted by Xin et al. (2008) assessed the toxicity of gas phase ethyl formate with respect to adults of three common durable commodity pests (*Sitophilus oryzae* (Linnaeus), *Rhyzopertha dominica* (Fabricius) and *Tribolium castaneum* (Herbst)) in an empty glass desiccator. Endpoint mortality was achieved within 24 hours of exposure. The results of the study were confounded by the absence of grain within the desiccator, negating the ability to determine sorption effects and effect on pre-emergent life stages. Another study conducted within a glass desiccator, examined the sorption effects of grain on the fumigation treatment of the above three pests, reporting a positive relationship between the quantity of grain and the required dosage of fumigant (Damcevski and Annis, 2006).

Sorption effects are the result of grain being a microporous product, with numerous microscopic channels throughout its structure (Banks, 1990). In relation to a fumigation treatment, these channels operate as a semi-permeable membrane, allowing small molecules to pass through, whilst restricting larger ones. A thorough understanding of gas behaviour is, therefore, imperative to the formulation and implementation of successful fumigation methods (Banks, 1990). This knowledge is a necessity given the fumigant concentration

experienced by a pest during a fumigation treatment is a determining factor for evaluating its efficacy (Banks, 1990). The diffusion and sorption effects of grain on ethyl formate fumigation are yet to be adequately evaluated, despite their importance to treatment outcomes (Banks, 1990).

This study investigated ethyl formate's capacity to control all life stages of three common grain pests of *S. oryzae, R. dominica* and *T. castaneum* within a simulated grain silo environment over an extended time period. The hypothesis of this study was that application of the liquid fumigant ethyl formate would result in complete mortality of the entire insect life cycle (eggs, pupae, larvae, adults) in two acute treatments. Results of this preliminary study will aid in determining if ethyl formate is a viable alternative fumigant and if it merits further investigation for more widespread application in the agricultural sector and farm bins.

4.2. Materials and Methods

4.2.1. Insect pest species used to test ethyl formate toxicity

Sitophilus oryzae (rice weevil), *Rhyzopertha dominica* (lesser grain borer) and *Tribolium castaneum* (red flour beetle) were selected as the test species as they provided a broad coverage of the pest beetle families commonly detected in stored grain. In eastern Australia, both *S. oryzae* and *R. dominica* have developed strong resistance to phosphine (Cousins, 2021).

This study used mixed-age cultures (including eggs, larvae, pupae, adults) of each test insect species. The tested cultures of the insect species of *S. oryzae, T. castaneum* and *R. dominica* were MUSO8, MUTC8/MUTC-SR-1 (strong phosphine resistant strains from Western Australia)/ MUWRD-HF-WR-2 (strong phosphine resistant strains from Queensland) respectively, held at the Post-Harvest Plant Biosecurity Laboratory, Murdoch University, Australia. Insect culturing and handling generally followed the techniques described by

Winks (1982) for *T. castaneum*. All mixed-age cultures of the test species were established by adding adults (400-500) to media (1 kg) at 25°C and 65% relative humidity (RH) for 4-5 weeks, by which time there were representative numbers from each stage – egg, larva, pupa, and adult – based on knowledge of development rates (Howe, 1952; Beckett et al., 1994). *S. oryzae* were reared on wheat. *T. castaneum* were reared on a medium comprising 1-part yeast and 12-parts wholemeal flour milled from Australian soft wheat (cv. Rosella). *R. dominica* were reared on media containing 40-parts wheat and 1-part wholemeal flour. Wheat and wholemeal flour were conditioned to 12.5% moisture content (m.c) (wheat and wholemeal flour) and disinfested by freezing at -20°C for more than 2 days prior to grinding or use for rearing.

4.2.2. Measuring sorption of ethyl formate on grains

The concentration of ethyl formate in each gas sample port (Figure 4-1) was determined on a Varian 3400 GC (Varian Instruments, Sunnyvale, CA), equipped with a flame ionisation detector (FID), after separation on a 50 m × 0.53 mm ID, GS-Q column at 140 °C and carrier flow (N_2) of 6 mL/min at 10 psi. Injection volumes of gases were 60 µL at timed intervals. Ethyl formate were calculated on the basis of peak areas against external standards.

1.1 L conical flasks (Bibby Sterilin, Staffordshire, Cat. No. FE 1 L/3) equipped inlet system with septa (Alltech Cat. No. 95326) were used for preparation of ethyl formate gas standards. The measured volume of each conical flask and was calculated from the weight of water required to fill the container and was used for calculations. Diluted gas standards were prepared by first removing the same volume of air as the known volume of concentrated fumigant to be injected into a conical flask (1.1 litre) containing 2 glass beads (2-3 mm o.d).

4.2.3 Design of simulated silo, and fumigation procedure

Fumigation was conducted in a small-scale simulated grain silo filled with wheat grain and steel cylindrical cages with mesh at either end (50 mm × 30 mm) containing mixed aged cultures of *T. castaneum*, *S. oryzae* and *R. dominica* were placed at various points within the grain bulk (Figure 5-1). The total pre-emergent immature life stages and adult insects (*S. oryzae* = 1495; *R. dominica* = 3438; *T. castaneum* = 1786, tallied 24 hours post-fumigation) were used. Immature life stages develop inside individual pieces of grain, consequently it was not possible to know the precise insect numbers at the outset of the experiment. The silo was a sealed PVC model, (1.6 m × 23 cm) with a capacity of 50 kg at 95% wheat capacity. Six gas-sampling ports were situated in various locations around the outside of the silo (Figure 4-1). A recirculation system for air and fumigant flow ran from the top of the silo through the grain to the bottom, with a flow rate of one air exchange per hour.

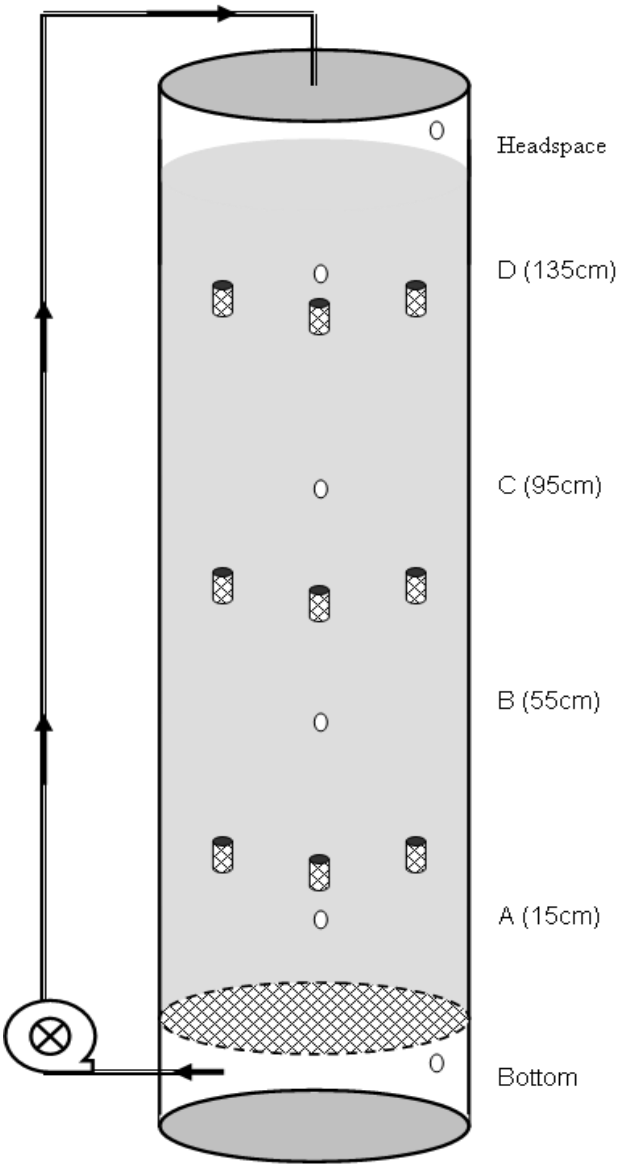

Figure 4-1.Schematic representation of a 52-55 kg (capacity) PVC silo, gas sampling ports (O), insect cages (⊠), ethyl formate application and re-circulation system.

Wheat used as the media in the silo had a moisture content of 11.3%, in keeping with general industry guidelines for grain storage, which stipulates that the commodity maintain a moisture content below 12%. Moisture content above 12% allows bacteria and fungi to thrive, compromising the integrity of the commodity (Emery, 2017).

The fumigant used was analytical grade (99.9%) liquid formulation of ethyl formate supplied by Aldrich, Sydney, Australia. The first application of 5 mL liquid ethyl formate (85 mg/L)

was made at the bottom of the mini silo, with recirculated air flowing at a rate of 1.2 L/hr, allowing the compound to be dispersed throughout the media. A second 5 mL dose of liquid ethyl formate was applied four hours later following the same protocol.

An un-fumigated control condition was run simultaneously to the simulated silo. The control (un-fumigated insects) was housed in a separate, controlled atmosphere container; containing pre-emergent immature life stages and adult insects (*S. oryzae* = 381; *R. dominica* = 1418; *T. castaneum* = 640, tallied at 24 hours post-fumigation) and media. The control conditions were identical in temperature and atmospheric conditions to the treatment. Mortality assessment protocols performed were identical to those from the simulated silo.

4.2.4. Determining insect mortality

Tallying of deceased and living adult insects was performed to assess acute mortality (24 hours following exposure to the fumigant) in both the treatment insects within the silo and the control insects. For an estimation of chronic mortality, emergence of adults from the wheat grains was assessed for the treatment and control insects at 1-, 3-, and 5-weeks post-fumigation, equating to pupal, larval and egg life stages (respectively) at the time of fumigation. This sampling regime was formulated based on previous studies of the life cycle of each species (Ren et al., 2008; Ren et al., 2012).

4.2.5. Measurement of temperature and relative humidity during the experiment

Temperature and relative humidity were routinely monitored throughout the study using a HOBO® data logger unit (Model number H08-004-02, Onset Computer Corporation, MA 02532, USA, http://www.onsetcomp.com) inside the simulated silo and control container. The silo and control container were both housed in a temperature and humidity controlled laboratory environment. Data recordings were analysed using BoxCar® Version 3.6+ for

Windows (Onset Computer Corporation, MA 02532, USA, http://www.onsetcomp.com). The HOBO®s were calibrated in the laboratory against each other and a standardised mercury-in-glass thermometer, as well as a range of glycerol/water solutions to maintain relative humidity.

4.2.6. Data analyses

Acute toxicity to ethyl formate for the three species of adult insects was determined based on the mortality rate observed in adult insects 24 hours post-fumigation. Emergence of adult insects at weeks 1, 3 and 5 post-fumigation were compared with the control. These emerging adults were immature life stages inside the wheat grain at the time of fumigation. Expected values for emerging adults in the fumigation treatment were calculated based on the life stage ratios of the controls using the initial adult counts in the fumigation treatment. Above emerging insect counts were divided between three locations within the silo (top, middle and bottom) in order to investigate if location within the silo influenced dispersal of fumigant and subsequent insect maturation and emergence from the wheat grain. Due to the preliminary nature of the trials, differences in mortality rates among treatments and species were subject to qualitative comparison, with the trial to be repeated on a larger scale if ethyl formate was found to be effective as a post-harvest fumigant.

4.3. Results

4.3.1. Sorption of ethyl formate on grains

Sorption of ethyl formate on grains was measured as disappearance of ethyl formate in each gas sample port (Figure 4-1). The loss of the ethyl formate in the mini silo was measured at six sample ports at timed intervals during the fumigation and plotted as the ratio (C/Co) of concentration (C) to the applied concentration (Co) against time. The mean of these concentrations are shown in Figure 4-2. After two hours of recirculation, the concentrations

of the ethyl formate were evenly distributed in the silo of wheat with a variation less than 10% (relative standard deviation). The concentrations of ethyl formate declined rapidly within the first 4 hours, particularly within the first 2 hours after application. Almost 80% ethyl formate was absorbed by the grain, but still remained above 10 mg/L of ethyl formate in the silo.

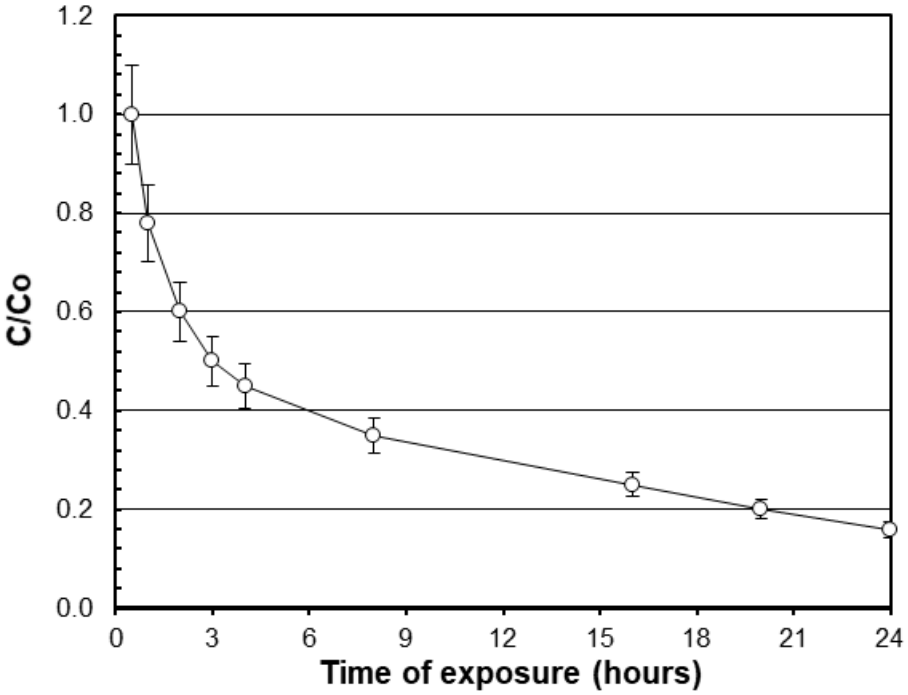

Figure 4-2. Sorption of ethyl formate on wheat, taken from measurement of loss of concentration in intergranular air at 25 °C. Sorption curve was formed with average concentration of ethyl formate from each sampling port, error bars indicated that standard deviation between each sampling port <10%.

4.3.2. Adult insect mortality following fumigation

Complete adult mortality (100%) was achieved 24 hours post-fumigation with ethyl formate for *R. dominica* and *T. castaneum*, with only slightly lower mortality (99.2%) for *S. oryzae* (Table 4-1).

Table 4-1.Acute mortality (%) in adults of three stored insect pest species 24 hours post-fumigation.

Species	Treatment	Live	Dead	Mortality (%)
R. dominica	fumigation	0	3438	100.0
	control	1418	0	0.0
S. oryzae	fumigation	12	1495	99.2
	control	379	2	0.5
T. castaneum	fumigation	0	1786	100.0
	control	640	0	0.00

4.3.3. Response of non-emergent insect life stages to ethyl formate fumigation

Variation in the pattern of emergence of adult insects from the grain was observed between the fumigation treatment and control (Figure 4-3). Pupae and eggs of *S. oryzae*, and the pupae of *R. dominica*, exhibited lower observed and expected rates, and different patterns of emergence of adults in the treatment relative to the control (calculated based on the life stage ratios in the control insects) (Figure 4-3). *T. castaneum* exhibited lower rates of emergence than expected, though not to the same extent as *S. oryzae*. Unlike *S. oryzae*, *T. castaneum* maintained a general pattern of emergence of insects in the treatment that was consistent with that exhibited by the control (Figure 4-3).

Position within the silo influenced emergence of adults in *T. castaneum* at three weeks post fumigation (larval life stage at the time of fumigation). A greater abundance of adults emerged in the top of the silo than in the middle or bottom regions (Figure 4-4).

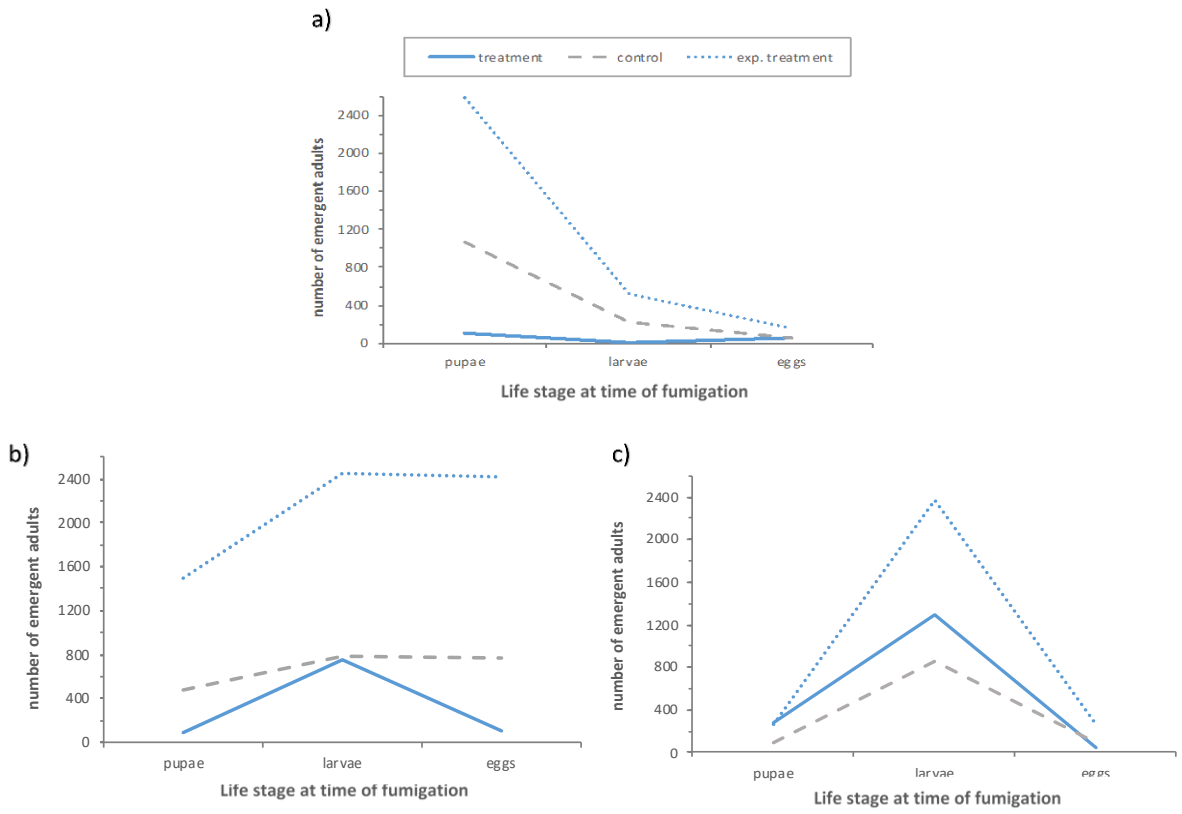

Figure 4-3.Number of emergent adult insects in the fumigation treatment and control at 1-, 3- and 5-weeks post-fumigation, corresponding to pupal, larval and eggs life stage at the time of fumigation. a) = Rhyzopertha dominica, b) = Sitophilus oryzae, c) = Tribolium castaneum. Immature life stages were within the wheat grain at the time of fumigation. Expected values for the treatment were calculated based on the life stage ratios evident in the control insects (standard deviation compared with mean is < 8%). NB: 'exp. treatment' = expected treatment

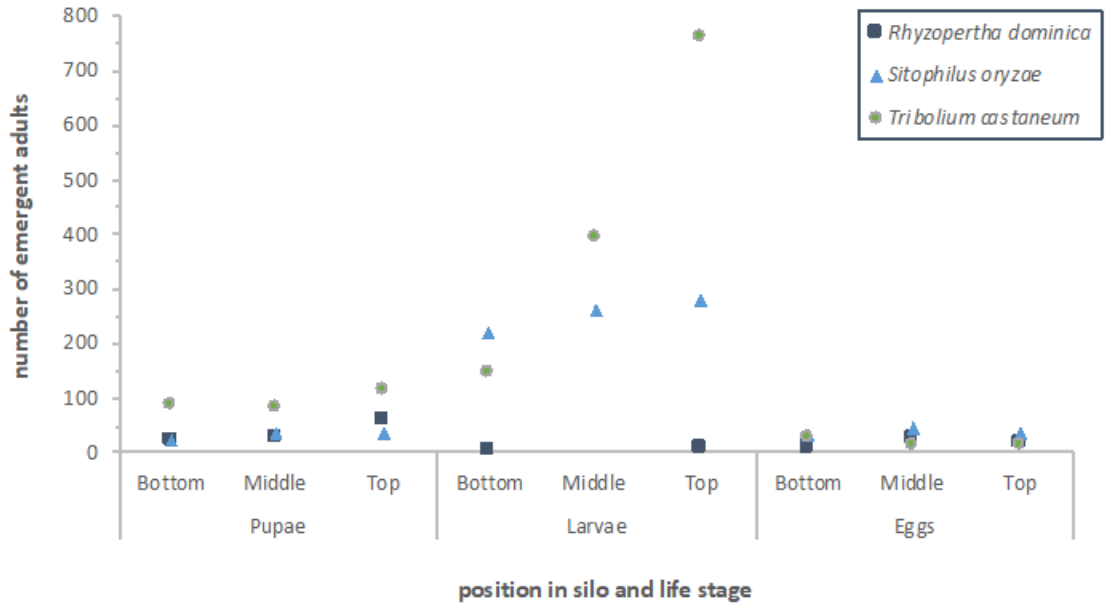

Figure 4-4. Number of emergent adult insects in the fumigation treatment for three insect species at three locations within the silo (top, middle and bottom) at 1-, 3- and 5-weeks post-fumigation (corresponding to pupal, larval and eggs life stage at the time of fumigation). Immature life stages were within the wheat grain at the time of fumigation). Standard deviation compared with mean is < 8%.

4.4. Discussion

Ethyl formate was highly toxic to the adult life stage as observed 24 hours post-fumigation for all three pest species assessed. All pest species exhibited an acute reaction, with greater than 99% adult mortality (100% mortality in *R. dominica* and *T. castaneum*). This acute effect is in keeping with the rapid action and strong penetration properties of ethyl formate. Ren and Mahon (2006) reported similar results, with all species examined in that study experiencing acute mortality greater than 99% relative to the control. That study was however, performed without the presence of media. Bin trials (50-55 t) containing wheat and

pea revealed that an ethyl formate application rate of 90 mg/L for 48 h exposure was effective in controlling all life stages of *T. castaneum* and *R. dominica* (Desmarchelier et al. 1998; Ren et al. 2003). Similar to my findings, results from that study indicate that ethyl formate is able to maintain its efficacy despite the introduction of a media. Given the similar mortality effects reported by the above studies, ethyl formate has the potential to be an effective fumigant with other adult stored commodity insect pests.

These gas concentration data was consistent with typical sorption curves, that is, the loss of fumigant from the gas phase followed the expected pattern, with an initial rapid sorption giving way to a long-term trend about 8 hours after dosing. This result was also consistent with results from previous trials with ethyl formate on wheat, barley, oats and peas (Desmarchelier et al., 1998, Ren and Mahon 2003 and 2006). This study found that the sorption of ethyl formate on peas is stronger than on wheat, barley, oats and canola.

Although ethyl formate was highly toxic to adults of all species, the fumigant was unable to induce complete mortality in the eggs, larvae and pupae of the three species studied, evident by the emergence of adults from the grain in the treatment silo at 1-, 3- and 5-weeks post-fumigation. This may have been a result of the limited sorption of ethyl formate into the grain or higher tolerance to ethyl formate of immature life stages. There was, however, some sorption of ethyl formate into the grain, evidenced by the lower comparative rates of adult emergence in the fumigation treatments across all species relative to the control, particularly in the pupae and eggs of *S. oryzae* and the pupae of *R. dominica*. The chronic effect of ethyl formate on life stages within the grain across all species contrasts with that reported by Ren and Mahon (2003), in which EF was determined to have a minimal effect in controlling of *S. oryzae* pre-emergent life stages. Although ethyl formate was observed to have a chronic effect on pre-emergent life stages in this study, it is likely that application of follow up doses to overcome the rapid breakdown of EF would be required in order to induce complete

mortality across all life stages (Muthu et al., 1984). Future investigations of ethyl formate should therefore examine the effects of follow up applications of EF in the weeks post initial fumigation.

The egg life stage of *S. oryzae* was the most susceptible to ethyl formate across the life stages of the three species examined, relative to the control. *S. oryzae* had a comparable number of adults at the start of the experiment compared to *T. castaneum*, and half that *of R. dominica*. As such, the lower observed numbers of adults emerging at 5 weeks post-fumigation (egg at the time of fumigation) may indicate that this life stage in *S. oryzae* is more susceptible to ethyl formate than the egg stage of the other two species. For susceptible species, higher mortality at various life stages will impact on the capacity of the population to recover to pre-fumigation numbers. Recovery of pest insect populations following fumigation has not been assessed in previous ethyl formate bioassays and would be a useful avenue of investigation.

Position within the silo may have an influence on the efficacy of ethyl formate for some species. For *T. castaneum*, higher adult emergence was recorded in the top section at week three (larvae life stage at the time of fumigation) compared to the middle and bottom. This may have been the result of variations in the circulation of the fumigant within the silo, which were potentially sufficient to cause small scale variability in fumigant concentration and hence its capacity for sorption. Future directions of research should include an understanding of the flow patterns of ethyl formate within storage containers.

A limitation of this study was the inability to distinguish between a lethal concentration of ethyl formate, and the existence and/or role of sorption effects post-fumigation. To allow for a better understanding of the use of ethyl formate as a fumigant, it would be beneficial to have continual concentration product data to allow for modelling of the results; (Xin et al. (2008), allowing for an evaluation of the relationship between pest mortality and fumigant

concentration. Future work could also measure gas concentrations in desiccators with and without grain to gain an understanding of how much the ethyl formate in the head space is reduced by sorption.

Overall, this study found that ethyl formate was highly effective in inducing acute mortality in the adult life stages of three species of stored-product pests. Emergence of adults from the grain in the weeks following fumigation indicated, however, that ethyl formate was not able to entirely eliminate the immature life stages (pupae, larvae and eggs) within the grain. Whether this was due to limited sorption of ethyl formate by the grain or higher resilience of immature life stages is not clear and warrants further investigation. Ethyl formate did however appear to induce a chronic effect on the immature life stages in the grain at the time of fumigant exposure, evidenced by the lower-than-expected rates of adult emergence across most life stages (based on estimates of life stage ratios in the controls). This reveals the potential of this fumigant in controlling stored product pests, particularly if initial fumigation is followed up by subsequent applications.

4.5. Conclusions

Results of this preliminary study are pertinent given the decline in effective treatment modalities for post-harvest pest control for durable commodities, particularly for managing the risk of phosphine resistance in grain insects. Finding alternative rapid fumigants is particularly important as the global populations grow, placing an increasing strain on agricultural supply markets to meet the demand for food and work towards food security. Given ethyl formate is potentially to offer a safe and readily biodegradable durable commodity fumigant, this study has implications for the agricultural industry and in a wider biosecurity and food security context. Further studies to assess the viability of ethyl formate

to control of other common insect pests are required and to ascertain if higher doses can be used to control all stages of pest insect lifecycles.

Chapter 5: Ethyl formate inter-reaction with electronic equipment and other contact materials

5.1. Introduction

Australia imports large quantities of products that are vulnerable to pest infestation, including motor vehicles and computers (valued at $23 and $8 billion per annum, respectively) (Ports Australia, 2019). The large number of products entering Australian ports exposes the nation to exotic insect pests that have potential to impact Australia biodiversity and agricultural commodities (Department of Water, Agriculture and the Environment, 2021; Sardain et al., 2019).

Each year, losses in crop production due to invasive insect pests cost the Australian agricultural industry $4.7 billion (Bureau of Rural Sciences, 2007). Many of the most serious insect pests are referred to as 'hitchhikers' (contaminating pests) and are associated with commodities that include no host material (Inspector-General of Biosecurity, 2018). The importation of cars, various electronics and farm machinery presents a serious biosecurity issue (Ross, 2005; Ormsby, 2018). Currently, sulfuryl fluoride and methyl bromide are the only approved fumigants for the quarantine treatment of inbound shipping cargo into Australia (Inspector-General of Biosecurity, 2018). Sulfuryl fluoride, an inert and highly unreactive compound, is the most widely used biosecurity treatment due to the fumigant's negligible effect on products and commodities (Derrick, 1990; Ormsby, 2018). Sulfuryl fluoride is however a potent greenhouse gas, and with methyl bromide being phased out around the world, there is an urgent need for an alternative fumigant (Muhle et al., 2009, United Nations Environment Programme, 2006). Prior to a new fumigant being deployed as a quarantine/biosecurity treatment, it is imperative to evaluate possible negative side-effects that may occur via the chemical reacting with the materials or equipment present inside the fumigation environment.

This is evident for fumigants such as phosphine, which has been documented to corrode metals such as gold and copper, resulting in it being unsuitable for biosecurity treatments of

electronics and motor vehicles (Hou et al., 2016. On the other hand, Methyl bromide, an approved biosecurity treatment, has been documented to corrode rubber, making it unsuitable for the treatment of motor vehicles (Bond and Monro, 1984). Whilst methyl bromide is thought to be safe for the treatment of consumer electronics, research conducted by Serre (2014) found that when applied in very high concentrations (300 mg/L), the fumigant was capable of negatively effecting the function of desktop computers. These factors pose a unique challenge to fumigation scientists. As such, there is need for an alternative fumigant that is not only effective and environmentally friendly, but also safe for use on vulnerable shipping cargo.

Ethyl formate has been investigated as a possible alternative fumigant (Ren and Mahon, 2006). Ethyl formate is an ester formed when ethanol reacts with formic acid, with a low molecular weight (74.1) and boiling point (54.3 °C) (Desmarchelier et al., 1999). The compound is soluble in water with some hydrolysis and mixable with various alcohols and aldehydes (National Center for Biotechnology Information, 2021). Ethyl formate is also unstable in high heat environments and readily undergoes hydrolysis to formic acid and ethanol (National Center for Biotechnology Information, 2021). Ethyl formate has also been found to react with nitrates, strong oxidizers, strong alkalis and strong acids (National Center for Biotechnology Information, 2021). Samarov et al. (2019), evaluated ethyl formate solubility, critical states and liquid-liquid equilibrium (LLE) in the quaternary reacting system (formic acid – ethanol – ethyl formate – water) and in the ternary subsystems (formic acid – ethyl formate – water and ethanol – ethyl formate – water) at 25 °C and 35 °C and atmospheric pressure (Maya et al., 2019); finding that the ternary subsystem will occur in dry matrix-based system such as fumigated grain and general cargo with ethyl formate. Despite these challenges, in the last decade, ethyl formate has been successfully used as a fumigation treatment for invasive pests in cereals and a variety of fruits, such as bananas (Krishna et al.,

2005), grapes and citrus (Ryan and De Lima, 2014). The rise of ethyl formate as a fumigant is largely a result of its fast action, strong penetration and degradation to biogenic substances ethanol and formic acid (Muthu et al., 1984; Ren and Desmarchelier, 2002). The threshold limit value for ethyl formate is 100 ppm, compared to 3 ppm and 0.3 ppm for methyl bromide and phosphine respectively. As such, ethyl formate can be regarded as 33 and 333 times less toxic respectively than these primary fumigant competitors (Agarwal et al., 2015). Because of the comparatively low human toxicity of ethyl formate, it can be considered as a safer fumigant from both an occupational safety and environmental standpoint (Coetzee et al., 2021).

A previous study by Rajendran (2001) evaluated the inter-reaction between ethyl formate, when used as fumigant, and a variety of materials. The results of this study reported that ethyl formate, when degraded to ethanol and formic acid, was capable of corroding unpainted iron and steel (Rajendran, 2001). The effects of ethyl formate on electronic componentry were not investigated.

Our current understanding, and generalisations regarding the use of fumigants, are unable to predict the specific reaction between gas and commodity. Consequently, the fumigant must always be tested with all materials it will encounter for accurate fumigation procedures to be decided (Bond and Monro, 1984). Due to ethyl formate's documented breakdown to ethanol and formic acid (a highly corrosive substance) via hydrolysis, it was imperative to evaluate if temperature / relative humidity conditions encountered in biosecurity treatments would facilitate this reaction (Mata-Segreda, 2000).

The aim of this study is to assess the effects of ethyl formate fumigation on electrical componentry. The study will firstly perform a preliminary fumigation of materials used to construct graphics processing unit (GPU). The GPU was chosen as a surrogate for the high

value and sensitive electrical componentry that is widely imported into Australia. Secondly this study will fumigate GPU's and compare the effect of ethyl formate with that of methyl bromide on its operational capacity.

5.2. Materials and Methods

All fumigation procedures were conducted at the Post-harvest Biosecurity and Food Safety Laboratory at Murdoch University between April and October 2020.

5.2.1. Fumigated commodities

For the preliminary evaluation of ethyl formate's inter reaction with the raw materials used in electronics, the following samples were fumigated: mild steel, high carbon steel (4140), stainless steel (316), aluminium (6160), copper, polycarbonate, acrylic, nylon, polyurethane, polyvinyl chloride and silicon rubber.

Nvidia Geforce GT710 graphics processing unit was selected for use in this study for evaluation of ethyl formate's effects on the operational capacity of electronics due to its representation of a wide array of components used in products that undergo biosecurity fumigations. These commodities include mobile phones and computers, as well as the electrical componentry used in new motor vehicles, which are increasingly focused on electronic componentry for function.

The compact size of the GT710 allowed these units to be placed within a small scale simulated controlled atmosphere environment (<10 L); whilst the three connection ports (DVI, VGA, HDMI) on the GPU provided the fumigant the greatest chance for internal penetration.

Figure 5-1. Graphics processing unit selected for fumigation with ethyl formate and methyl bromide.

5.2.2. Fumigation Protocol

Preliminary fumigation was undertaken in a fumigation chamber (47.8 cm x 40 cm) constructed from steel with a net volume of 60 L. For recirculation, the chamber was equipped with Swagelok compression fittings for attachment to a diaphragm pump just above the bottom rim and just below the top rim of the drum. The removable lid was fitted with a centrally located septum fitting for introduction of gas during fumigation. The fumigation chamber was fitted with 2-4 gas sampling ports which were located on the side of the fumigation chamber. Each lid was fitted with gas-tight seals which were pressure-tested before use. Pressure halving time of the fumigation chamber was greater than 3 min (Figure 5-2).

Plastic and metal materials (Table 5-2) were placed in the chamber and ethyl formate was injected into the container at a concentration of 90 mg/L, with an ambient temperature of 25

ºC at 56-63% RH. The exposure period was six hours, and at the end of the fumigation period, the container top was opened to vent ethyl formate into the atmosphere for 30 minutes. This fumigation was only performed once as a preliminary investigation.

Figure 5-2. The fumigation chamber (60 L) used for preliminary investigation of ethyl formate inter reaction with materials, equipped with gas injection and sampling systems.

Operation capacity fumigations were conducted in a small-scale controlled atmosphere environment, representative of a 20-foot shipping container encountered in biosecurity treatments. For this study, two airtight 9.9 L desiccators (designated A and B) were used to house the GPU. A 1.1 L conical flask was used as the gas standard (GPU absent) to allow to for the calculation of the gas concentration in desiccators A and B. A control (unfumigated) GPU was also placed in a separate desiccator in the same temperature and relative humidity conditions to allow for fair comparison with treatment GPU.

Figure 5-3. Conducting fumigation of graphics processing unit using desiccator (9.9 L) as the fumigation chamber with ethyl formate and methyl bromide.

Two temperature/humidity conditions were replicated in specialised climatic chamber (model HWS, Ningbo Southeast Equipment CO. Ltd.), designed to emulate typical conditions encountered within a shipping container that could potentially impact the integrity of electric commodities during fumigation. The first condition was representative of typical ambient temperature and relative humidity, 25 °C and 60-65% relative humidity. The second condition was representative of higher heat conditions, 40 °C with relative humidity between 60-65%. These conditions encourage breakdown of the fumigant, providing the greatest opportunity for the GPU to be damaged by the chemical.

In both conditions, the fumigation desiccators containing the GPU, conical flask and desiccator containing the control GPU were stored within the temperature and humidity-controlled chamber for 18 hours prior to fumigant injection. Following injection of the fumigant, each desiccator was removed for gas sampling at scheduled sampling times, before being promptly returned to the chamber. Following the relative fumigant exposure period, all

desiccators were removed and ventilated inside a fume hood at a laboratory at 23-25 °C and approximately 60% relative humidity.

Two fumigants, ethyl formate and methyl bromide, were used at each of the temperatures. Sample size (n) was 4 for each fumigant-temperature pair, and n=2 for controls at each temperature/humidity; to a total n=20 (**Error! Reference source not found.**).

Table 5-1. Experimental setup for fumigant treatments and graphics processing unit sample size.

Temperature and humidity	**Control (no fumigant)**	**Ethyl formate**	**Methyl bromide**
25^{o}C at 60-65% relative humidity	N=2	N=4	N=4
40^{o}C at 60-65% relative humidity	N=2	N=4	N=4

5.2.3. Ethyl Formate

Food grade liquid ethyl formate (>97%) was sourced from Sigma Aldrich, Sydney, Australia. A 6-hour fumigation protocol was used at a dosage of 90 mg/L for preliminary and operational capacity trials. The dosage of ethyl formate applied was calculated using Equation 5-1.

$$\text{Dosage } (mL) = \frac{\text{Concentration } (mg/L) \times \text{Volume (L)}}{\text{Purity (\%)} \times \text{Density } (mg/mL)} \quad \text{.....Equation 5-1}$$

Prior to the dosage being applied, 300 mL of air (equivalent to the dosage volume of ethyl formate gas) was removed from the test condition chamber, and 30 mL from the 1.1 L conical

flask to avoid changes in pressure. For the test condition, 1 mL of liquid ethyl formate was injected via two deliveries of a 0.5 mL syringe into the desiccator (9.9 L). For the control condition, 0.1 mL of liquid ethyl formate was injected into the conical flask (1.1 L) using a 0.5 mL syringe. The liquid ethyl formate was delivered onto a filter paper placed inside the desiccator acting as an evaporating surface to absorb and accelerate volatilisation of the solvent. Following injection of ethyl formate, gas sampling was performed at 10 minutes, 1, 3 and 6 hours. For each gas sample, 60 µL of gas was drawn using a 100 µL gas tight syringe, and then immediately injected into the gas chromatograph.

5.2.4. Methyl Bromide

Methyl bromide (>98%) was sourced from BOC gases Australia. A 24-hour fumigation protocol was used a dosage rate of 48 mg/L applied in keeping with the Australian Government standard for methyl bromide fumigation (Australian Quarantine and Inspection Service, 2008). The dosage was calculated using Equation 5-2.

$$Dosage\ (mL) = \frac{Concentration\ (mg/L) \times Volume\ (L) \times gas\ constant(22.414\ mL)}{Molecular\ Weight\ (mg/mmol) \times Purity\ (\%)} \text{.....Equation 5-2}$$

As liquid methyl bromide had to be transferred into a compressed bottle, to then be applied in gas phase, the Avogadro constant had to be used in the dosage calculation (Equation 4-2).

For the fumigation trials, 300 mL of air was removed from each 9.9 L desiccator (test condition), and 30 mL from the 1.1 L conical flask (control chamber) to avoid pressure changes. For the test condition, 113 mL of methyl bromide was injected using a 100 mL and 25 mL gas tight syringes. For the control condition, 12.6 mL of methyl bromide was injected using a 25 mL gas tight syringe. 60 µL of gas was drawn periodically (10 minutes; 1, 3, 6 and 24 hours) using a 100 µL gas tight syringe. Gas sampling was extended to 24 hours to reflect the longer exposure period of the fumigant as done by industry (Australian Quarantine and Inspection Service, 2008).

5.2.5. Measurement of fumigant concentrations

During the fumigation process, ethyl formate and methyl bromide gas concentrations were determined using an Agilent 6890 gas chromatograph equipped with a flame ionisation detector (FID). Separation was achieved on a HP-5 Column (J&W Sci., Agilent Technologies, Santa Clara, CA; 19091J-413), with the oven at a temperature of 100 °C, with injector and detector temperatures at 250 °C. The 60 µL gas samples were manually injected into the gas chromatograph, and the concentrations were calculated based on peak areas against the calibrated ethyl formate and methyl bromide gas standards. Gas standards were monitored periodically during the respective 6- and 24-hour exposure periods.

5.2.6. Measurement of GPU performance following fumigation

Assessment of GPU performance was conducted one-week post-fumigation to allow for any detrimental corrosive effects to take place. It was also done to assess if there were any acute effects following chemical exposure. Subsequent testing was conducted at six months post-fumigation to assess if there were any chronic effects on performance following chemical exposure.

To assess GPU performance, the GPUs were connected to a testing personal computer located inside the Post-harvest Biosecurity and Food Safety Laboratory. Heaven benchmarking software (UNIGINE Corp) was used to stress test and measure the performance and stability of the GPU. The benchmarking software executed a standardised series of demanding graphical processing tests. The testing involved comprehensive use of adaptive hardware tessellation. The benchmarking was running in full screen mode (1024×768) with 8x anti-aliasing and provided outputs that were entirely bound to the performance of the GPU. Frame rate, or frames per second, was used as the key performance indicator for analysis.

Each GPU was run through the benchmarking program three times at each time point (one week and six months post-fumigation). The three benchmarking tests were run back-to-back to exert maximal stress on the GPU.

5.2.7. Data Analysis

Data were analysed quantitatively using SPSS (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). A one-way repeated measures analysis of variance (ANOVA) and Tukey HSD for separation of means was used to assess for differences among treatments (temperature and fumigation, and controls) based on GPU performance output at the time points. Descriptive statistics were also performed, providing the mean for the GPU frames per second scores.

Gas concentration graphs were generated in Microsoft Excel (Microsoft Corporation. Microsoft Excel. 2011. Version 1902).

5.3. Results

5.3.1. Preliminary fumigation

Following fumigation, visual evaluation with the naked eye was performed on each treated material in comparison with an untreated control 24 hours post exposure. Visual analysis did not identify any tarnishing of the material's colour or damage in comparison with the untreated control.

Figure 5-4. Photographs of fumigated and unfumigated materials from preliminary ethyl formate inter-reaction investigation.

Materials	**Treatment and control materials 24 hours post fumigation**	
	Treated	**Untreated control**
Mild Steel		
Copper		
Polycarbonate		
Aluminium		
Acrylic		
Nylon		

Materials	Treatment and control materials 24 hours post fumigation	
	Treated	**Untreated control**
Polyurethane		
Stainless Steel (316)		
High Carbon Steel (4140)		
Polyvinyl Chloride		
Silicone Rubber		

5.3.2. Gas Chromatography-Mass Spectrometry method for analysis of ethyl formate and methyl bromide

The fumigant gas samples were analysed during the fumigation period with Gas Chromatography-Mass Spectrometry (GCMS). The spectra showed that ethyl formate was detected and completely separated with Gas Chromatography-Flame Ionisation Detector at 0.5 ppm, with no breakdown compounds (ethanol and formic acid) being detected. Therefore, it is a reliable method to indicate break down materials of ethyl formate, particularly formic acid which is metal corrosive substance (Figure 5-5). Methyl bromide was very stable during

the fumigation period, with GC spectrum showing the signal peak. The Mass Spectrometry spectra showed that methyl bromide was identified at very low levels (0.2 ppm) (Figure 5-6).

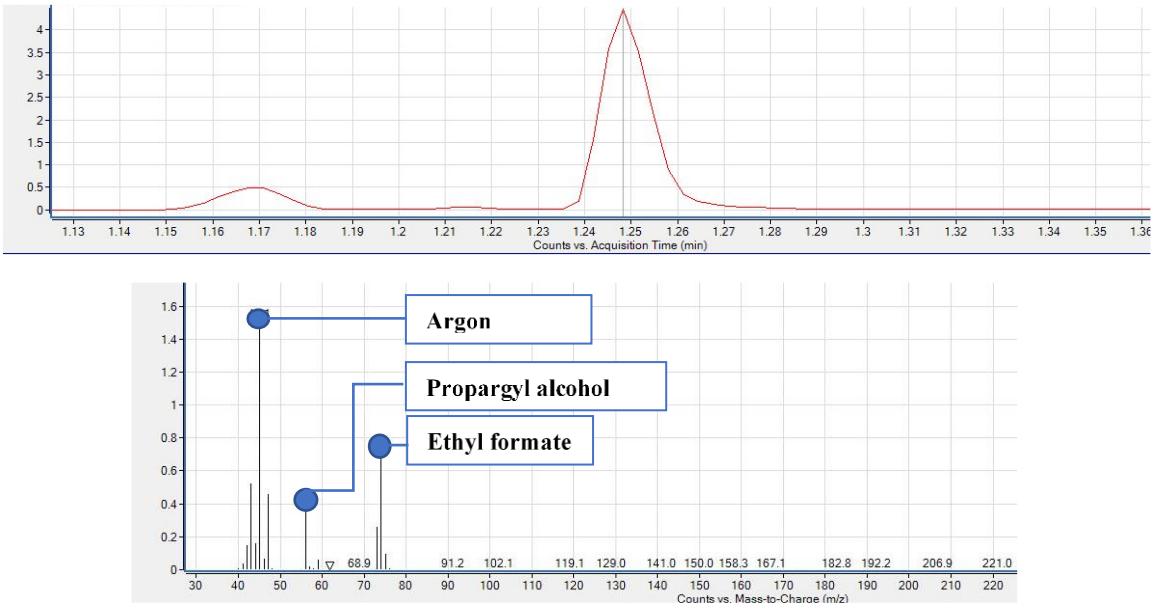

Figure 5-5. Gas Chromatography and Mass Spectrometry signal peaks for ethyl formate and identified compounds from analysis.

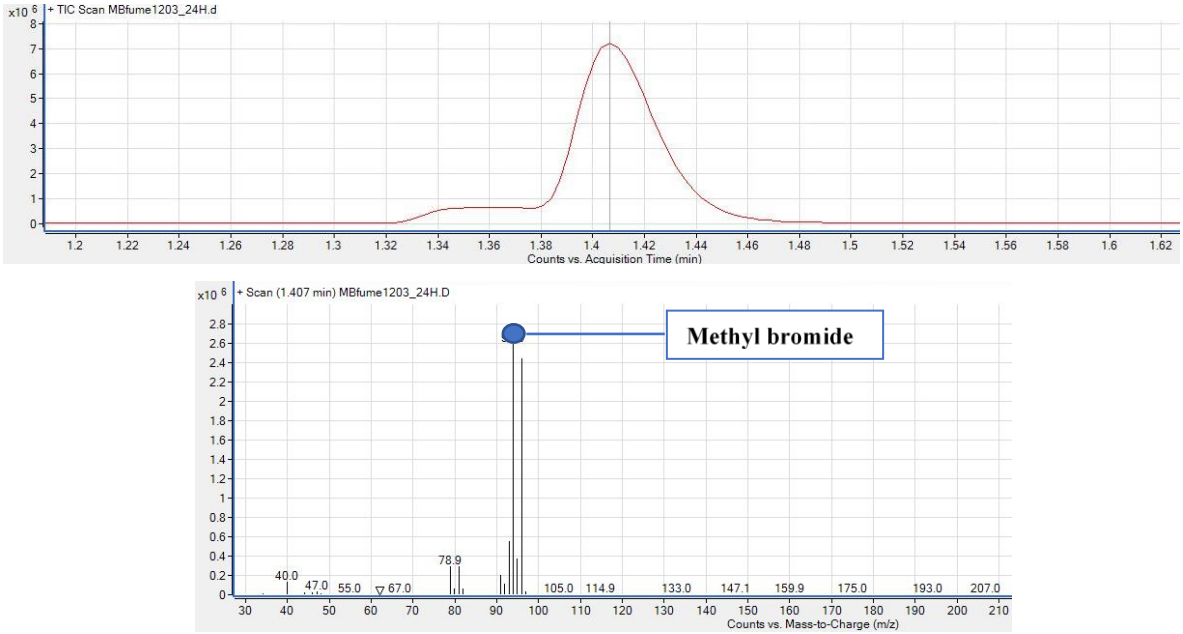

Figure 5-6. Gas Chromatography and Mass Spectrometry signal peaks for methyl bromide and identified compounds from analysis.

5.3.3. Ethyl formate

The time (10 minutes, 1, 3 and 6 hours) by treatment (25 °C, 40 °C) interaction for ethyl formate was not significantly different (*P*=0.359).

According to Levene's test, there was homogeneity of variance among the gas concentration curves across the time points.

A test for among-subjects effects found overall mean concentrations were not significantly different (*P*=0.941). The mean overall gas concentrations for each treatment were as follows: 40 °C (90.43 mg/L), 40 °C standard (89.98 mg/L), 25 °C (89.38 mg/L) and 25 °C standard (90 mg/L).

Sphericity was tested using Mauchly's W, which indicated there was equality of variance among mean gas concentrations (*P*=0.138).

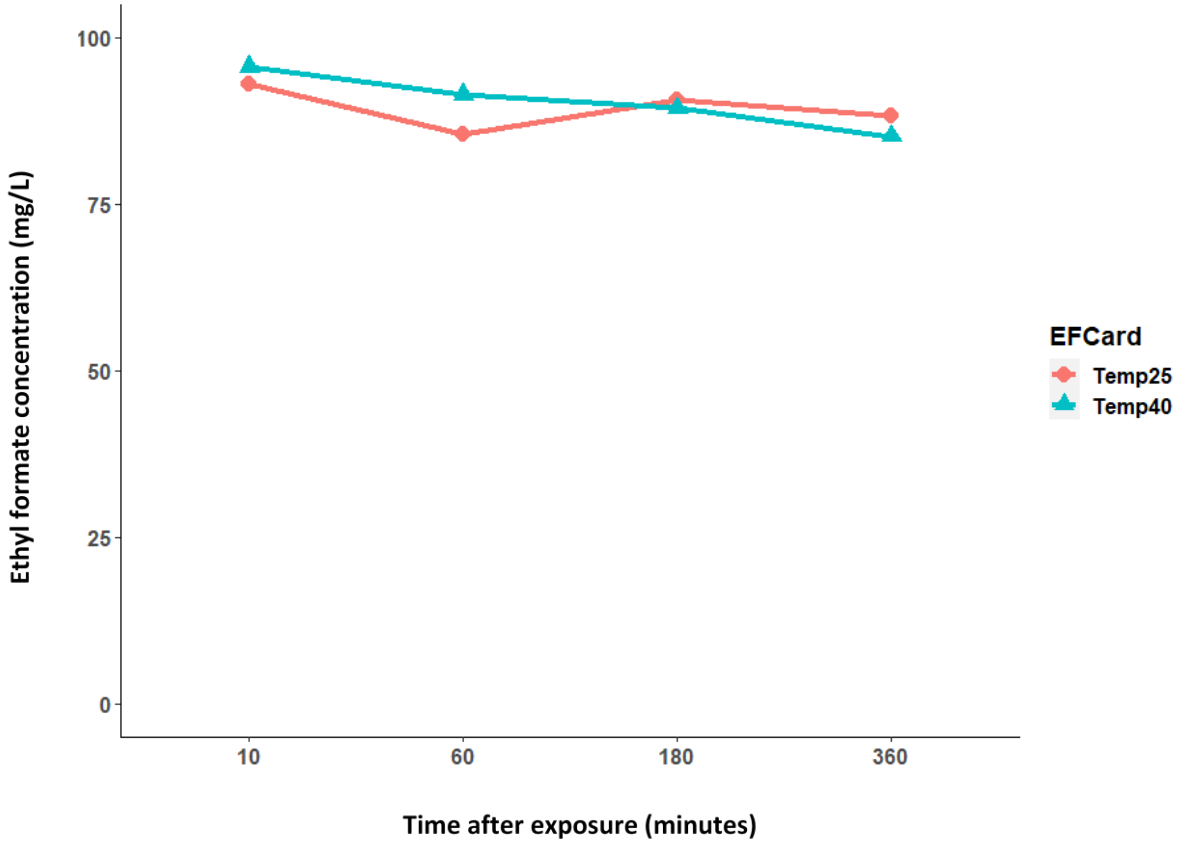

Figure 5-7. Mean gas concentration across 6 hours exposure time for all graphics processing units fumigated with ethyl formate (variation of gas concentration compared with mean is < 8%).

5.3.4. Methyl bromide

For methyl bromide, the time (10 minutes, 1, 3, 6, 24 hours) by treatment (25 °C, 40 °C) interaction followed a linear trend and was not significantly different (*P*= 0.219).

According to Levene's test, gas concentrations were not homogenous among treatment, with 10 minutes (*P*= 0.02) significantly different 3 hours (*P*= 0.016).

Overall mean MB concentrations were significantly different among-treatments, irrespective of time point effects (*P*= 0.001). Tukey's HSD *post-hoc* test determined that the 40°C GPU treatment had a significantly lower mean gas concentration (43.59 mg/L) compared to gas

concentrations for the 40°C standard (46.62 mg/L), 25°C (48.17 mg/L) and 25°C standard (47.66 mg/L).

Sphericity was tested using Mauchly's W, which indicated there was equality of variance among mean gas concentrations (*P*= 0.071).

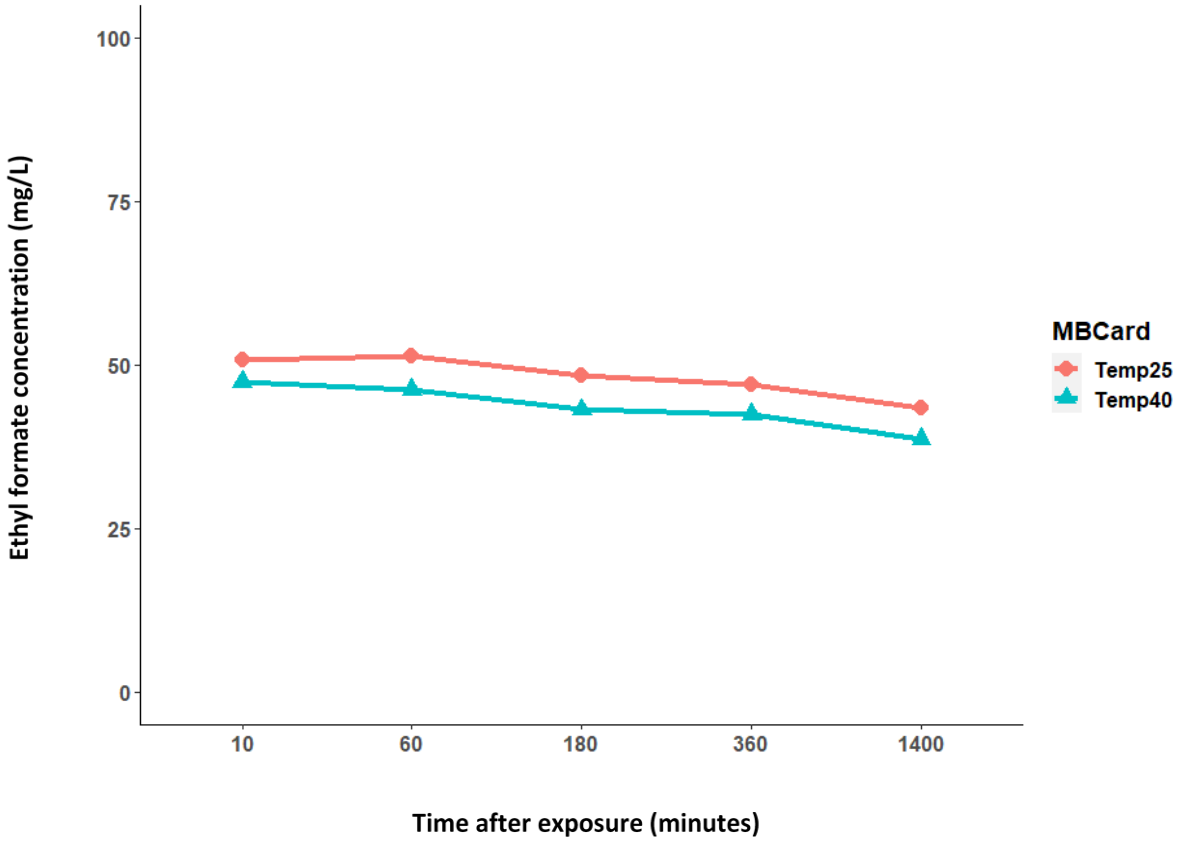

Figure 5-8. Mean gas concentration across 24 hours exposure time for all graphics processing units fumigated with methyl bromide (variation of gas concentration compared with mean is < 8%).

5.3.5. GPU post fumigation analysis

Visual analysis was performed by the same person each time post-fumigation to identify signs of corrosion on GPUs. Surface observation did not show any diminishing or corrosion of the metal.

GPU performance testing following fumigation provided results in the range of 8.3 to 8.4 frames per second (Figure 4-6). An ANOVA was performed on the results and showed no significant difference between fumigant (*P*= 0.6624).

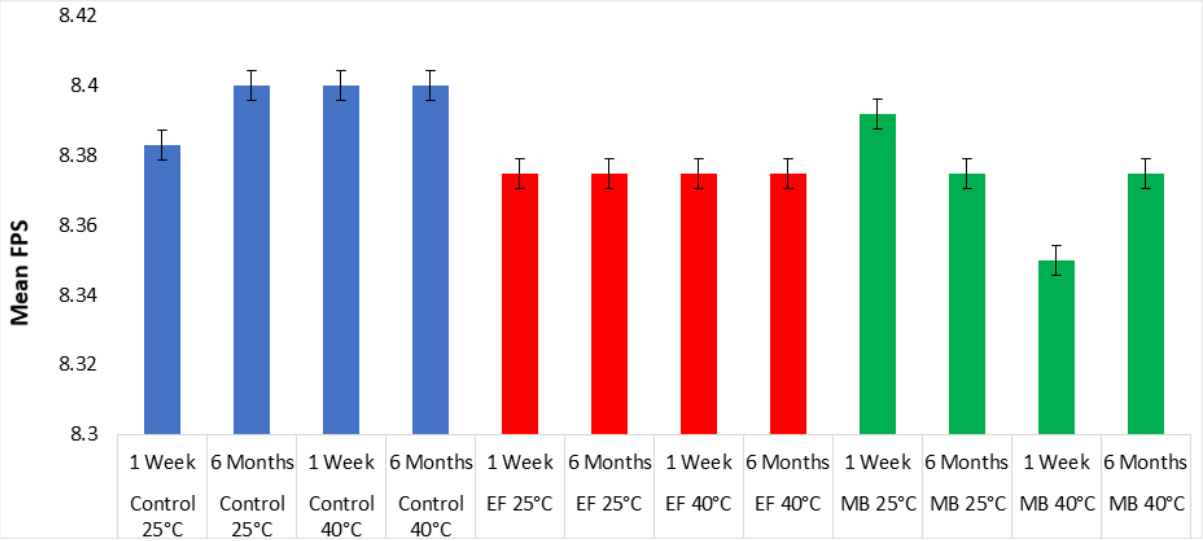

Figure 5-9. Mean (±standard error) frames per second for GPU fumigated with different fumigant, temperature (°C) and time post-fumigation. The mean score was calculated from three benchmarking tests of each graphics processing unit. MB=methyl bromide; EF=ethyl formate.

5.4. Discussion

High economic value is associated with many commodities imported into Australia, including motor vehicles and other electronic goods (Ports Australia, 2019). While seemingly not suitable hosts for insect pests, these products are vulnerable to infestation by known pest species, such as the BMSB, and require treatment to mitigate the risk of pest incursion events associated with importation of these items (Department of Agriculture, Water and the Environment, 2021a). While treating imported cargo of this nature, it is not only imperative that quarantine fumigation treatments are sufficiently effective to eradicate any pests present, but also that the treatments do not affect the functioning and integrity of the products, or their appearance. Previous bioassays using ethyl formate on BMSB found that the fumigant was effective in controlling the insect (Kawagoe et al., 2017; Chapter 3 of this thesis). This study indicates that use of ethyl formate for the fumigation treatment of delicate electronic items, such as the circuit boards in motor vehicles and other electronic goods, may provide a viable, more stable and environmentally friendly alternative to use of methyl bromide.

This study reports that the fumigant ethyl formate was more stable at higher temperatures compared to methyl bromide, based on concentration curves. There was no significant difference among treatments for ethyl formate at the two values of temperatures and humidity tested (25 °C and 40 °C, 60% RH). This result is consistent with Tarakanova and Yukhnevich (2013), who reported that water plays a very important role in the proton solvation of ethyl formate and the products of EF's hydrolysis. As there was no free water present in the fumigation chamber, ethyl formate could not readily degrade to ethanol and formic acid.

In contrast, methyl bromide concentration curves varied significantly among treatments with the mean concentration for the 40 °C treatment significantly lower than at 25 °C. This may potentially indicate that ethyl formate is a more stable fumigant at higher temperatures compared to methyl bromide, and therefore a more reliable biosecurity treatment. This

stability is in line with ethyl formate's higher boiling point (54.3 °C) compared to methyl bromide (3.56 °C) (National Center for Biotechnology Information, 2021). Research conducted by Bond and Monro (1984) supports the findings of this study, where solvent boiling point has an inverse relationship with volatility. The fumigant concentrations in this study are those used as industry standards. As such, the results are commercially applicable.

The GPU benchmarking tests indicated that neither ethyl formate nor methyl bromide had a noticeable effect on performance of the GPU compared to the control. Despite the differing exposure times (in line with industry standards), both ethyl formate and methyl bromide are liquid chemicals that are vaporised to gas and are therefore a fair comparison. Interestingly, our results for GPU performance following fumigation with methyl bromide contrast with previous evaluations of the MB fumigation of electronic components (desktop computer), where function was negatively affected (Serre, 2014). However, the concentrations documented by Serre (2014) were radically higher than the regulatory mandated concentration of 48 mg/L used in this study (Australian Quarantine and Inspection Service, 2008).

Results from this study indicate there is limited interaction between ethyl formate and certain electronic components, and products likely to be encountered in quarantine fumigations. During the period tested, ethyl formate did not negatively affect GPU performance, and is the performance of GPU were comparable to the control and those fumigated with methyl bromide. Despite the fact that the results presented here are not reflective of the interaction between ethyl formate and all electronic commodities that will be encountered during quarantine fumigations, which warrants further investigation, there is indication that EF may be a viable biosecurity treatment alternative for vulnerable electronic products.

5.5. Conclusions

This study indicates that ethyl formate is a safe fumigant to use on commodities that include GPUs and other sensitive electronic components. We would expect similar results to be recorded following use of ethyl formate in other products, including computers, mobile devices and vehicle electrics.

The biosecurity implications of pest incursion and establishment have the potential to impact social, environmental, economic and cultural values of affected countries. Pest treatments, such as fumigation, must be implemented to reduce the biosecurity risk countries are exposed to when receiving goods. At the same time, biosecurity managers implementing pest treatments must be cognisant that treatments may have potential negative consequences on the environment and/or on the goods themselves, particularly electronic components and contact materials. Results of this research highlight the benefits that can be achieved by an expanded use of ethyl formate as a pest treatment fumigant.

Chapter 6: General discussion and conclusions

6.1. General discussion

Crop production accounts for half of Australia's agricultural industry, with an annual contribution to the economy of approximately $37.5 billion (Australian Bureau of Agricultural and Resource Economics and Sciences, 2022). Crop production also directly employs in excess of 73,000 Australians, including farmers, labourers and fruit pickers (Labour Market Information Portal, 2022). Over the past 20 years, the gross value of Australia's agricultural production has increased by 7% (adjusted for inflation); however climatic modelling based on a rise in temperatures and drop in rainfall predicts profitability could be negatively impacted by 22.6% (Australian Bureau of Agricultural and Resource Economics and Sciences, 2022). This leaves very little room for further impediments to the profitability of crop production in Australia such as that which may be caused by invasive insect pests. In 2002 invasive insect pests were estimated to cost the Australian economy at least $8 billion annually and it would be safe to conclude that this cost has continued to increase (Canyon et al., 2002).

In an increasingly globalised world where international trade is central to the economy, biosecurity inspection and surveillance cannot be solely relied upon to safeguard Australia's plant and environmental resources from the risk of new invasive insect species. An ever-growing plant biosecurity threat to Australia, and the globe, is invasive insect pests, that hitchhike on imported goods or in sea containers. The movements of sea containers continue to increase with traffic in 2021 exceeding 240 million twenty-foot equivalent units (World Shipping Council, unpublished data). Addressing the hitchhiking of pests in sea containers needs to be a priority for governments and ultimately biosecurity treatments of inbound shipping, such as fumigation, are at the vanguard of safeguarding Australia's plant industries from invasive insect pests.

The first step in my research was to conduct a novel risk analysis case study to see what the specific costs of poor biosecurity could be for a plant industry should an invasive insect pest successfully enter, spread and establish. A case study using the Australian wine industry was undertaken, with modelling of the potential costs associated with brown marmorated stink bug (BMSB) establishment. The wine industry (wine grapes specifically) was selected because it allowed for an assessment of the potentially large costs and losses inflicted upon a relatively small (2%) component of the Australian agricultural industries (Australian Bureau of Agricultural and Resource Economics and Sciences, 2022). BMSB was chosen as the invasive insect pest for this analysis as it is designated in Australia as a top 10 national priority invasive insect pest (Department of Agriculture, Water and the Environment, 2019). The BMSB is also regarded as a hitchhiking pest, infesting new countries by stowing away in miscellaneous freight, cargo and sea containers whilst overwintering (Ormsby, 2018). An example of the threat posed by BMSB is its effect in the United States, which has seen it detected in 42 states, and devastate (>90% losses recorded) horticultural crops in the mid-Atlantic region since its arrival in 1996 (Hahn et al., 2016; Leskey et al., 2012). Of particular concern in the United States have been the difficulties in eradicating BMSB, which has largely been ineffective, raising concerns over insecticide efficacy and increasing insecticide resistance (New Jersey Agricultural Experiment Station, 2022). As a result, the key lesson learnt from the United States is that not only is the BMSB highly destructive to horticultural crops, but once established it is very difficult to eradicate. As a result, it is imperative to provide industry and government with the hard data to make the most informed biosecurity decisions.

The risk/ financial analysis I undertook was based on a conservative 5-15% loss in vineyard yield. Whilst the analysis parameters were on the low end of the spectrum for

potential damage, they did reveal that BMSB was still capable of costing the industry $45 million per year. When taking into account currently established pests and diseases that cost the Australian wine industry $251 million annually, the additional costs from BMSB could be crippling (Grape and Wine Research and Development Corporation, 2010). The analysis undertaken in this thesis presents decision makers with clear information on the financial risk industry could face, and therefore the importance of continuing to implement strict biosecurity measures to prevent BMSB from entering Australia. The other key outcome was identifying the potential increase in the level of BMSB damage over time, providing further information to industry and government about how easy it is for the BMSB threat to be underestimated due to the minor damage costs for the first decade whilst the insect population establishes. Case in point is the effects of poor biosecurity, which allowed BMSB to establish in the United States, with inaction from regulators, who listed the insect as 'non-actionable/non-reportable' up to 2010, allowing population numbers to radically increase (Holtz and Kamminga, 2010). Based upon these results, prevention of BMSB establishment via effective biosecurity treatments is the best option.

The currently approved biosecurity treatments for inbound shipping into Australia are heat treatment and fumigation using methyl bromide or sulfuryl fluoride (Australian Chief Plant Protection Office, 2020). Each of these treatments has been documented to be effective in controlling BMSB under ideal conditions (Kuhar and Aigner, 2016; Ormsby, 2018; Abrams et al., 2020). However, biosecurity treatments are rarely performed under ideal conditions, and the cold winter temperatures of Europe have seen heat treatment exposed as an unreliable treatment modality due to repeated failures (Associated Customs and Forwarding, 2019; Department of Agriculture, Water and the Environment, 2021b). Whilst methyl bromide and sulfuryl fluoride have been shown as consistently effective treatments for BMSB, they each possess significant drawbacks that threaten their long-term viability.

For instance, methyl bromide is gradually being phased out due to its status as an ozone depleting substance, and sulfuryl fluoride is an extremely potent greenhouse gas (Park et al., 2020; Papadimitriou et al., 2008). Both fumigants are highly toxic to humans, and there are concerns regarding their safety (Baur et al., 2015; Shipping Australia Limited, 2019). Due to these problems, alternative fumigants are under investigation, with ethyl formate being the focus of this thesis. Ethyl formate's low toxicity to humans, rapid action and food grade status has meant that it possesses great potential as a biosecurity treatment and there was a need for further research to support the approval and implementation by government regulators. Ethyl formate has been approved by the Australian Government through a Minor Use Permit as a treatment against hitchhiker pests in sea containers being sent to Barrow Island (Australian Pesticides and Veterinary Medicine Authority, 2021) but it currently not approved and a general treatment for our scenarios. As a progression from the Minor Use Permit, this study sought to evaluate the efficacy of ethyl formate for wider application, focusing initially on BMSB.

The ethyl formate bioassay discussed in Chapter 3 sought to determine the fumigant's applicability for the control of BMSB. The results found that ethyl formate was highly effective in controlling BMSB irrespective of dormancy state or temperature. The key finding from the laboratory trial was that not only was ethyl formate twice as effective at controlling diapausing BMSB compared to sulfuryl fluoride, but it was also achieved in a quarter of the exposure time (3 hours vs 12 hours) reported by Abrams et al. (2020). The short exposure time is highly advantageous logistically; and only having to use a small amount of a chemical with already low environmental and human toxicity further enhances the benefits of ethyl formate as an alternative fumigant. This was the first-time ethyl formate has been evaluated for the control of BMSB at multiple dormancy states and temperatures. The findings of this study will provide government and regulators with the

confidence to deploy ethyl formate as a biosecurity treatment for BMSB irrespective of the dormancy or temperature condition encountered.

The chapters of the thesis to this point successfully demonstrated the real potential impact of BMSB to an Australian plant industry, the efficacy of ethyl formate as a biosecurity treatment to kill BMSB hitchhiking in imported products and the safety of using the fumigant on a range of sensitive cargoes. The fourth chapter of this thesis considered ethyl formate as a biosecurity treatment for exported products. Given that approximately 72% of Australia's agriculture production is exported, there was a necessity to evaluate ethyl formate's capacity to control insect pests that may negatively affect the integrity of the commodity or breach the trading partner's phytosanitary requirements. The grain industry was the focus of the final component of the research, due to its size and importance to the Australian economy, accounting for 26% of agricultural production, with exports worth $10.3 billion per annum (Australian Bureau of Agricultural and Resource Economics and Sciences, 2022; Brown, 2022). A key biosecurity consideration for the importance of an effective fumigant is the federally mandated zero tolerance of live insect pests in exported grain (Cousins, 2021). With phosphine being the only registered grain fumigant available, and insect resistance becoming more common, it presented a key opportunity for ethyl formate, a food grade substance to be evaluated as a potential alternative (Xin et al., 2008). Given that up to one third of grain crops worldwide are lost in post-harvest storage due to insect pests (Emery and Cousins, 2019), it was an important final bioassay to determine efficacy of ethyl formate on stored grain pests (*S. oryzae*, *T. castaneum*, *R. dominica*) .This study was particularly important given that all three insect species tested have been documented as displaying varying levels of resistance to the primary stored grain fumigant, phosphine (Cousins, 2021). The primary outcome for this study was that ethyl formate is effective in controlling adult insects across all three species and was unaffected by the

presence of grain in a simulated silo. As the first study to report these findings, this is very important information for industry and regulators as it shows the effectiveness of the fumigant goes beyond a controlled laboratory bioassay. The emergence of adult insect's post fumigation showed that multiple applications of ethyl formate over time was necessary to control all life stages. This finding is critical as it provides information to industry and regulators on fumigation protocols going forward with ethyl formate to ensure maximum treatment efficacy. This study showed that ethyl formate has the capacity to be an effective stored grain treatment, with the timing of multiple applications the primary hurdle to ensuring complete insect control.

Finally, a key consideration before application of any treatment it whether or not it will react negatively or affect the product or commodity. It is well known for example that phosphine will corrode gold, silver and copper, thereby making it unsuitable for biosecurity treatments in many circumstances (Hou et al., 2016). This is highlighted by Australia's reliance on the importation of motor vehicles and computers (valued in excess of $30 billion) that are vulnerable to many chemicals including Phosphine. It is imperative that ethyl formate is systematically evaluated for its suitability as a treatment to be used on vulnerable cargo (Ports Australia, 2019) before widespread implementation.

In Chapter 5, I evaluated potential inter-reaction (damage or impairment of function) between ethyl formate and materials and electronic componentry potentially encountered in or with imported commodities. This study was the first to report on the potential effects of ethyl formate fumigation on the function of electronic componentry and will provide valuable information to biosecurity managers seeking to implement the use of a safer and more environmentally friendly alternative to other commonly used fumigants. The materials (various metals and plastics) and graphical processing unit's fumigated with ethyl formate displayed no evidence of corrosion or functional impacts. This clearly indicated that ethyl

formate is suitable for application in the vicinity of the aforementioned materials/products in a biosecurity setting without causing any damage. The findings of this study were the first to be reported for ethyl formate, and further enhance the confidence of government and regulators in deploy it for biosecurity treatments.

6.2. Conclusions

With increasing movements of international cargo, impacts of climate change and greater cost pressures on Australia's agricultural industry, the management/control of invasive insect pests which threaten the viability of industries and the economy as a whole has never been more important. Whether it is safeguarding Australia's crop production from the arrival of new invasive species, or protecting valuable grain exports from insect infestation, ethyl formate has demonstrated that it has to capacity to play a major role in future management. Ethyl formate was rigorously evaluated against nationally identified insect pests in a variety of environmental, controlled atmospheric and insect dormancy/resistance conditions. Ethyl formate clearly produced strong results in each study conducted and has shown its superiority to currently used biosecurity treatments in multiple facets. This thesis has also shown that Australia, and indeed the world cannot afford to be complacent with the current biosecurity treatments when there is so much economically at risk to invasive insect pests. In conclusion, ethyl formate is a safe, reliable and effective fumigant that needs to be approved by government regulators globally as a front-line biosecurity treatment.

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