The efficacy of propylene oxide, ethyl formate, and ethanedinitrile as fumigants to control
Lasioderma serricorne (Fabricius, 1792) (Coleoptera: Anobiidae), *Tyrophagus putrescentiae*
(Schrank, 1781) (Sarcoptiformes: Acaridae), and *Necrobia rufipes* (De Geer, 1775) (Coleoptera:
Cleridae)

by

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Abstract

It is estimated that 1/3 of harvested food is lost to stored product pests. *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae), and *Necrobia rufipes* (De Geer) (Coleoptera: Cleridae) are harmful pests to several high-valued stored products such as tobacco, dry-cured meats, and pet foods. Due to the phase-out of methyl bromide, the corrosive properties of phosphine, the increasing global populations of *L. serricorne* that are becoming resistant to phosphine, and the ineffectiveness of sulfuryl fluoride to control *L. serricorne*, *T. putrescentiae* or *N. rufipes*, there is a need for alternative fumigants that are effective at controlling these pests and reducing post-harvest losses. Propylene oxide (PPO), ethyl formate (EF), and ethanedinitrile (EDN) are used as fumigants to control several stored product pests. However, thorough studies to control *L. serricorne, T. putrescentiae,* and *N. rufipes* are lacking. Thus, laboratory experiments were conducted with propylene oxide, ethyl formate, or ethanedinitrile to evaluate the efficacy of each fumigant at controlling these pests.

PPO fumigation indicated that the most tolerant life stage of *L. serricorne* was the larval stage, with an estimated LC_{50} of 41.08 mg/L. The most tolerant stage of *L. serricorne* was the pupal stage, with an estimated LC_{50} of 28.14 mg/L when EF was used. *T. putrescentiae* eggs were the most tolerant life stage with an estimated LC_{50} of 0.87 mg/L PPO, and an LC_{50} of 19.74 mg/L EF, respectively. *T. putrescentiae* eggs were also the most tolerant life stage to EDN fumigation, with an estimated LC_{50} of 283.37 ppm. Time-response assays indicated complete mortality of *L. serricorne* larvae within 3 h at a concentration of 70.09 mg/L PPO. *L. serricorne* pupae were completely controlled within 3 h when 95.22 mg/L EF was used. Total mortality of

T. putrescentiae did not occur within 12 h when PPO was used at a concentration of 71.42 mg/L but did occur at 70.09 mg/L EF.

L. serricorne mixed life stages were controlled entirely for six weeks after a 24 h exposure of either fumigant at 25 °C ± 1 °C with 70% R.H. in 16:8 (L:D). *T. putrescentiae* mixed life stage fumigations yielded less than one mobile *T. putrescentiae*, emerging after 24 h exposure of 186.9 mg/L PPO at 25 °C ± 1 °C with 70% R.H. in 16:8 (L:D). Exposure of PPO at the same application rate provided complete control for *N. rufipes* and complete control of T. *putrescentiae* and *N. rufipes* after a 24 h exposure of 190.4 mg/L EF at the same conditions. Mixed life stage control of *T. putrescentiae* was achieved at an EDN concentration of 600 ppm, and less than 0.05% of the population survived after a treatment of 300 ppm within a 24 h treatment at 25 °C.

The sorption of PPO reduced mortality by 20%, 30%, and 80% in pet food, flour, and tobacco, respectively, for a 12 h exposure. There was a 10%, 30%, and 40% mortality reduction in pet food, flour, and tobacco when exposed to PPO for 24 h. There was no significant impact on mortality during a 12 h EF exposure; however, there was a 10% mortality reduction in tobacco with a 24 h EF exposure. Sorption of 93.5 mg/L PPO increased the survivability of *T. putrescentiae* mobile stages by less than 0.05% in fishmeal, ham, or pet food for 12 h and 24 h exposures. There was less than an 8% survivability increase for *T. putrescentiae* larvae and less than a 0.02% increase for *T. putrescentiae* mobiles in any of the selected commodities when exposed to 95.2 mg/L EF for 24 h or 12 h. Neither gas desorbed significantly enough to impact the survival of *T. putrescentiae* mobiles, *L. serricorne* larvae, or *L. serricorne* pupae in any of the fumigated commodities for either exposure time of 12 h or 24 h.

These studies support that alternatives such as PPO, EF, and EDN are effective at controlling *L. serricorne, T. putrescentiae,* and *N. rufipes* on select commodities. Future studies using commercially formulated products should be conducted to determine if they are also able to control these pests. Quantitative sorption and desorption assays on selected products of these pests and the addition of residual analyses would be an applicable approach to further support these alternative fumigants in receiving regulatory approval from government agencies as pesticides on commodities in the U.S.

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Dedication

I dedicate this thesis to the grandparents that were active in my life. My Papa Smith and his late wife, Nana Barb. I am so grateful for the summers spent during my childhood on your farm, where you both nurtured my curiosity and encouraged my love of entomology. My Grandma Cheryl for being there in times of great need and my Grandma Mary for the many enjoyable and lively discussions at your picnic table. Without the four of you, I could not be who I am today. Thank you!

Chapter 1 - Introduction and Objectives

Stored Product Fumigation

Stored commodities are any raw good that is used as an input in the production of goods and services. Stored products are postharvest agricultural commodities that have been differentiated with added value by a manufacturer or by branding (Lioudis 2019). The process in which a stored commodity becomes a stored product is an additional step of processing during the post-harvest chain. The post-harvest chain is multifaceted, including transportation, storage, processing, and end-market placement of each commodity. Stored product is the term in this review that will identify any commodity or product that is in the storage step of the post-harvest chain. Globally there are over 1,104 stored products reported and categorized into stored grains, legumes, fruits, nuts, processed products, and durable products (Hagstrum and Subramanyam 2009). Processed products are products that have been transformed from the raw commodity into a marketable item. Examples of processed products are flour, baked goods, pet foods, market-ready tobacco products, and spice blends. Durable products refer to products that are unique to the other categories, such as whole spices, raw tobacco, cured tobacco, mushrooms, seeds, and various animal products.

Stored products are subject to post-harvest loss, which accounts for 1.3 billion tons of worldwide commodity loss, or $1 trillion U.S. Most of this loss is directly attributed to pest infestation between the time of harvest and consumer' use (Buzby et al. 2014). Worldwide, 75% of all harvested crops can be lost before consumer purchase, equating to approximately $100 billion U.S. (Wacker 2018). Postharvest losses can be attributed to physical and biological attributes. Physical losses are caused by a commodity or product being destroyed through abrasion, puncturing, cracking, or transportation wear. Fungi, vertebrates, and invertebrates cause biological damage or loss to commodities. *Aspergillus*, *Fusarium,* and *Penicillium* are

three genera of fungi of biological concern in stored products, as they produce mycotoxins, which have detrimental toxic effects on consumers (CAST 2003). Rodents, birds, skunks, and raccoons are vertebrates that can transmit diseases, mechanically vector foodborne illnesses, and destroy commodities (Kells 2012). Invertebrates of concern include at least 1,663 insect species and 280 mite species that are associated with stored product infestation (Hagstrum and Subramanyam 2009; CAST 2003).

Invertebrate pests are classified as primary or secondary pests. Primary pests are internal feeders, completing their life cycle inside a commodity, which decreases profitability and nutrients. Secondary pests are external feeders and complete their life cycle outside the commodity while contaminating the stored product with frass, exuviae, and dead bodies (Hagstrum and Subramanyam 2009). Postharvest losses are suppressed through the implementation of integrated pest management (IPM) programs that are specific to the facility of storage and the pests commonly found in products of storage at their facility. Stored product IPM integrates sanitation, monitoring, and mitigation methods. A structured routine cleaning of a facility is necessary for successful sanitation. The use of traps can help monitor the pests that are present in the facility. Mitigation includes methods of biological control, physical control, and chemical control. Biological control includes insect pathogens, parasitoids, and predators to mitigate pest populations; however, this method is not well developed for stored product management. There are only about seven commercially available parasitoid or predator species available, and the U.S. Food and Drug Administration (FDA) has insect particle limits on stored products (FDA 2018b, White and Johnson, 2010). Pest exclusion, facility design, packaging design, extreme temperature treatments, modified atmosphere treatments, and controlled atmospheres are all methods of physical control. Physical control methods are usually employed

in the design of a facility or marketing of a product and are expensive to employ after a facility is built and operating. Structural sprays, grain protectants, surface treatments, aerosols, and fumigation are methods of chemical control that are heavily relied upon in stored product pest management systems to achieve disinfestation.

Fumigation is a method of chemical control applied with an application of a toxic gaseous chemical that suppresses pest populations or disrupts pests. Fumigation is used to prevent the economic loss of stored products and for purposes of quarantine. "Ideal" fumigants are highly volatile, denser than air, non-corrosive, penetrate materials, have minimal sorption, have high desorption rates, and with little or no residues left on the product (Bond 1989). Fumigant efficacy is dependent on the pest presence, the target species, the target life stage, the age of target life stage, the target species sex, temperature, pressure, concentration of application, fumigant formulation, and the insect's uptake of the fumigant (Phillips et al. 2012). Chiefly, insect fumigant uptake occurs by the toxicant entering through the spiracles by a concentration gradient. The toxicant then flows into the trachea, tracheoles, epithelial cells, and the hemolymph to reach the toxicant's target site. Minor fumigant uptake can occur orally from fumigant residuals and through cuticular penetration (Bond 1961).

The application of a fumigant is dependent on the facility, the product, the target pest, and the label of the registered fumigant. The U.S Environmental Protection Agency (EPA) and the U.S. FDA establish and enforce the laws of pesticide usage and tolerance of residuals for non-food commodities and food commodities (EPA 2016a, FDA 2018a). Many grains, legumes, and seed fumigations in the U.S. are conducted in storage bins with a recirculating or "closed-loop" system. This recirculating system draws the accumulated fumigant from the headspace and recirculates it to the sub-floor plenum of the storage bin forcing the fumigant to rise through

the commodity for consistent recirculating coverage (Jones et al. 2011). Products can be fumigated on railcars during transportation, under tarps, and in closed-off warehouses (Phillips et al. 2012). Compounds like ozone and carbon dioxide are registered as fumigants by the EPA; however, in this thesis, these fumigants will not be considered fumigants for chemical control, but rather as compounds used to modify or control facility atmospheres. Thus, this thesis will focus on synthetic chemical fumigants.

History of Synthetic Fumigation

Fumigation with synthetic compounds in commercial stored products was not readily accessible until the end of the 19th and the beginning of the 20th century. Four fumigants that were developed during that time were carbon disulfide, chloropicrin, ethylene oxide, and hydrogen cyanide. These historic fumigants are currently banned, used for quarantine in the U.S., or were forgotten about and are now being reconsidered as viable fumigants. Some historic fumigants received little use when methyl bromide fumigation became standard practice (Bond 1989). The phase-out of methyl bromide has contributed to increased use of phosphine and sulfuryl fluoride as fumigants of stored products.

Historic Synthetic Fumigants

The oldest fumigant used for insecticidal purposes was carbon disulfide (CS_2; carbon bisulfide), with its synthesis claimed by Doyère (1856). However, in 1854, Garreau independently published experiments with various compounds against grain weevils using a compound called "sulfure de carbone" or carbon disulfide (Garreau 1854). Doyère did not propose carbon disulfide for liquid application to stored products until 1857 (Doyère 1857). Goodwin gave credit to Doyère for introducing the material as an insect fumigant even though the synthesis and suggestion to be used as an insecticide was attributed prior to Doyère

(Goodwin 1916). Regardless of work established by Garreau (1854), Doyère (1857), and Goodwin (1916), W.E. Hinds' paper "Carbon bisulfide as an Insecticide" has been the standard reference for the first use of carbon bisulfide, now referred to as carbon disulfide, as an insecticide (Hinds 1917). Carbon disulfide is a heavy (density = 1.26 g/cm³ at 25°C) colorless, noncorrosive, noncarcinogenic, and a highly flammable liquid. During its use, carbon disulfide was not very toxic and used as a soil or space fumigant in warehouses. Explosions due to the unstable nature of carbon disulfide were frequent, which makes it challenging to use as a fumigant (Bond 1989; Fleming and Baker 1935). Carbon disulfide is banned in the U.S. for stored product pest control but has been reregistered in Australia and China since 2000 (TEAP 2000).

Another major historic fumigant is hydrogen cyanide (HCN; hydrocyanic acid). Hydrogen cyanide was called blue acid and was first extracted by Scheele in 1782 from the pigment Prussian blue $[Fe^{II}(CN)_6]_3$, which was used since 1706 (Scheele 1782, Frisch 1896). Gay-Lussac researched, named, and more thoroughly established properties of several cyanide compounds in 1815 (Gay-Lussac 1815). Historically hydrogen cyanide was used in World War I, World War II, and the Iran-Iraq War against the Kurds as a chemical warfare agent (Chauhan et al. 2008) due to its properties as a light (density = 0.6876 g/cm³) colorless gas (Bond 1989). Hydrogen cyanide was not established as an insecticide until Coquillett used it to control the San José scale in 1886 (Sullivan 1920). Hydrogen cyanide was used mainly in grain, legumes, seeds, and other dried products. This fumigant is very toxic, penetrates well, and leaves minimal residue (Woglum 1949).

The next fumigant used as an insecticide was chloropicrin (CCl_3NO_2; nitrochloroform, trichloronitromethane), which was first synthesized in 1848 by Stenhouse (Stenhouse 1848).

Chloropicrin did not have much attention until after World War I when it was used as a tear gas by the Germans (Anonymous 1918). Near the end of World War I, a U.S. scientist Moore (1917), and independently, the Italian scientists Piutti and Bernardini (1917) described chloropicrin as a broad-spectrum gas that was toxic to insects. Chloropicrin is a heavy (density = 1.69 g/cm^3), colorless gas that was reregistered in the U.S. for soil fumigation but is still unavailable for stored product fumigation (EPA 2019).

The last historic fumigant used as an insecticide is ethylene oxide (C_2H_4O; epoxyethane), which was first synthesized by Wurtz (1859). Ethylene oxide was recognized by the Americans and shared with the Ally powers as an essential compound in World War I to synthesize sulfur mustard gas (Chauhan et al. 2008). Ethylene oxide is a light (density = 0.8821 g/cm^3) colorless gas that was not shown to have insecticidal potential until 1928 (Bond 1989, Cotton and Roark 1928). Ethylene oxide was used in bulk grain, packaged foods, and tobacco and had intermediate toxicity to insects (Cotton and Roark 1928). Ethylene oxide lost registration when the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) amendment lapsed, but it is being reviewed for re-registration for uses other than soil (EPA 2016b, 2019).

Methyl Bromide

Methyl bromide (CH_3Br; bromomethane) is a dense (density = 1.73 g/cm^3) colorless, nonflammable, noncorrosive gas that was first used in grain as an insect fumigant (Goupil 1932, Bond 1989). Methyl bromide is a broad-spectrum intermediately toxic fumigant that was used for over 73 years in stored products, wood, and soil fumigation because it evenly diffuses throughout a commodity and space to achieve disinfestation with little to no residues (Bond 1989; Getzendarer et al. 1968; Savarie et al. 2005; Godfrey and Young 1943; Andrews et al. 1943). In 1992, methyl bromide was identified as an ozone-depleting substance due to its

reactive nature with ultraviolet waves, which releases atomic bromide, which in turn reacts with ozone to produce bromine oxide (EPA 2018a). Thus, it was included as an ozone-depleting substance in the international Montreal Protocol, which called for a phase-out of compounds with ozone-depleting properties (Oberthür 1997). The U.S. signed the Montreal Protocol treaty in 1988 and enacted the U.S. Clean Air Act in 1990, which classified methyl bromide as a Class 1 ozone-depleting substance.

In order to comply with the Montreal Protocol treaty and the U.S. Clean Air Act, methyl bromide is still used for quarantine, pre-shipment, and critical use exemptions (CUE) for California strawberry growers; however, exemptions are time-sensitive and are not guaranteed (EPA 2018a). Dry-cured pork products were given exemptions until 2018 and relied heavily on methyl bromide for disinfestation of pests due to a zero-tolerance of mite infestation on dry-cured ham products (EPA 2018a, EPA 2011). For example, methyl bromide was used 1-5 times per year in 34 dry-cured ham processing facilities surveyed in North Carolina, Virginia, Kentucky, Missouri, Tennessee, and Georgia. Twenty-two of these surveyed facilities used methyl bromide for mite control, and half of those facilities utilized methyl bromide to control red-legged ham beetles (Rentfrow et al. 2008). By 2014, less than 0.02% of the methyl bromide produced in the U.S. before 2005 remained (EPA 2018a). The lack of methyl bromide availability has disrupted dry-cured pork pest management with minimal feasible alternative fumigants available. Therefore, many other stored product managers increased the use of the registered alternative fumigants phosphine or sulfuryl fluoride to suppress pest populations effectively.

Phosphine

Gengembre discovered phosphine (PH3; phosphane) in 1783; however, it was not used as a grain fumigant until the 1930s to control stored product pests in Germany (Gengembre 1785, Heseltine and Thompson 1957, Fluck 1973). Phosphine has been used to fumigate cured tobacco leaves to control the cigarette beetle, *Lasioderma serricorne* (F.), in the U.S. since around 1960. Phosphine is currently registered for application to several stored products, including grains, pulses, nuts, oilseeds, dried fruits, and dried animal products with the maximum allowable phosphine residues of 0.01 ppm (Phillips et al. 2012).

There are two primary application forms for phosphine fumigation, which include gaseous and tablets. Phosphine tablets have been formulated of aluminum phosphide ($AlPH_3$) or magnesium phosphide (Mg_2PH_3) to react with water vapor to release phosphine over time. These two forms of phosphine application give stored product managers flexibility in application, cost options, and duration of potency (Phillips et al. 2012). A significant advantage of using phosphine is that it is highly penetrative and disperses quickly. Phosphine does not have ozone-depleting properties and decomposes into safe compounds after diffusing into the environment (Chaudhry 1997).

A growing disadvantage is global phosphine resistance because of the high selection pressure placed on insects repeatedly exposed to phosphine applications. Evidence for genetically inherited resistance to phosphine in several storage insect pests, include but are not limited to *L. serricorne* (Zettler and Keever 1994, Sağlam et al. 2015), *R. dominica*, (Collins 1998, Schlipalius et al. 2008, Chen et al. 2015, Schlipalius et al. 2018), *T. castaneum* (Collins 1998, Chen et al. 2015), *Sitophilus oryzae* (Schlipalius et al. 2018), and *Cryptolestes ferrugineus* (Schlipalius et al. 2018). For example, in 2003, 31 *L. serricorne* populations from 12 countries

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were resistant to phosphine, and by 2015, all U.S. *L. serricorne* populations tested were resistant to phosphine (Savvidou et al. 2003, Sağlam et al. 2015). Stored product managers are encouraged to minimize chemical control by utilizing IPM strategies and alternate the fumigants that are utilized. Additionally, phosphine is highly corrosive to copper, the primary conductor used in many electrical wirings, which makes it an undesirable fumigant for some storage, food processing, or marketing facilities (Bond et al. 1984). Dry-cured ham processors are reticent to use phosphine to fumigate ham aging rooms due to the presence of copper wiring in the HVAC system and processing equipment (Zhao et al., 2015). Resistance and corrosive properties have made some stored product managers switch to using sulfuryl fluoride.

Sulfuryl Fluoride

Sulfuryl fluoride (SO_2F_2; sulfonyl fluoride) was developed by Stewart in the 1950s as a structural fumigant to control the dry-wood termite, *Kalotermes minor* Hagen; additionally, it was approved for the disinfestation of stored products in 2005 for the U.S. as a methyl bromide alternative (Stewart 1957, Phillips et al. 2012). Sulfuryl fluoride is a heavy (density = 2.88 g/cm3) colorless broad-spectrum gas (Bond 1989, Baltaci et al. 2009). Sulfuryl fluoride is ten times more penetrating than methyl bromide, and it is non-ozone depleting (Rajendran 2001).

Sulfuryl fluoride releases a fluoride anion as a product of degradation and is regulated at between 0.01 ppm-15 ppm depending on the commodity that is fumigated (EPA 2018b). For example, sulfuryl fluoride is not an adequate alternative for *L. serricorne* as it is not registered for tobacco, and it is ineffective for controlling *L. serricorne* eggs (EPA 2005, Su and Scheffrahn 1990). Egg tolerance to sulfuryl fluoride is standard in many stored product pests and is likely due to the sulfuryl fluoride's impermeability of the pest chorion, which limits diffusion into the egg (Outram 1967). Therefore, sulfuryl fluoride is also ineffective at controlling *Tyrophagus*

putrescentiae eggs at room temperatures (Phillips et al. 2008). Unfortunately, the efficacy of sulfuryl fluoride is limited by high egg survival and fluoride anion residues.

Proposed Alternative Fumigants

Due to the phase-out of methyl bromide and the pitfalls of phosphine and sulfuryl fluoride, the demand to find alternatives that can suppress pest populations is necessary since many post-harvest losses are directly attributed to pest infestation (Buzby et al. 2014). Alternative fumigants would ideally be highly volatile, denser than air, non-corrosive, penetrating, have minimal sorption, high desorption rates, with little or no residues left on a product (Bond 1989). Alternatives that share these ideal attributes and lack of ozone-depleting properties yet being effective are desirable. Three fumigants that fit many of these ideal categories are propylene oxide, ethyl formate, and ethanedinitrile.

Propylene Oxide

Propylene oxide (C_3H_6O) has a molecular weight of 58.08 g/mol with a specific gravity of 0.86 and a boiling point of 34 °C, causing this fumigant to be liquid at room temperature and normal pressure. Propylene oxide is flammable at concentrations of 3-37% volume by volume (v/v) in air (Phillips et al. 2012). The flammability of this compound has been tempered with carbon dioxide, leading to its approval in the U.S. as a microbial sterilant (Griffith and Warren 2000). Propylene oxide has been used as a microbial sterilant since 1958 and was used as an insecticide until 1988 when re-registration under the FIFRA amendment lapsed (Griffith 1999, EPA 2016b). Propylene oxide is a relatively safe fumigant for food because it quickly degrades to propylene glycol and other diols (Griffith 1999). Propylene oxide has insecticidal properties that can control stored product pests including *T. castaneum* (Isikber et al. 2004, Navarro et al. 2004, Gautam et al. 2014), *T. confusum* (Griffith 1999), *T. variabile* (Griffith 1999, Zettler et al.

2003), *Plodia interpunctella* (Hübner) (Griffith 1999, Zettler et al. 2003, Isikber et al. 2004, Isikber et al. 2006, Gautam et al. 2014), *L. serricorne* (Gautam et al. 2014), *Carpophilus hemipterus* (L.) (Zettler et al. 2003, Gautam et al. 2014), *Amyelosis transitella* (Walker) (Gautam et al. 2014), *Ephestia cautella* (Guenée) (Isikber et al. 2004), and *Ephestia elutella* (Hübner) (Gautam et al. 2014).

Ethyl Formate

Ethyl formate ($C_3H_6O_2$) has a molecular weight of 74.08 g/mol, a specific gravity of 0.92, a boiling point of 54 °C, and it is flammable at levels of 2.8-16.5% (v/v) air (Phillips et al. 2012). In the U.S., ethyl formate is labeled as a "generally recognized as safe" (GRAS) compound and food additive by the FDA with an allowance of 0.01%-0.05% depending on the food item (FDA 2018a). Previously, ethyl formate was used for individual package fumigation of dry fruits in 1929 (Simmons and Fisher 1945) and was evaluated for grain protection in the 1980s (Muthu et al. 1984). Ethyl formate residues break down to the naturally occurring products, formic acid, and ethanol (Desmarchelier et al. 1998). The behavior of ethyl formate residues on different grains in Australia were studied by Desmarchelier et al. (1998), Ren and Desmarchelier (2001), and Vu and Ren (2004) in preparation for the approval of a commercial formulation for the disinfestation of postharvest pests on grains and additional commodities in Australia in 2005 (Ryan et al. 2006). Ethyl formate has been evaluated and found useful at controlling several stored product pests, including *S. oryzae* (Muthu et al. 1984, Damcevski and Annis 2002, Xin et al. 2008), *T. castaneum* (Muthu et al. 1984, Damcevski and Annis 2002, Xin et al. 2008), *R. dominica* (Damcevski and Annis 2002, Xin et al. 2008) and *Sitotroga cerealella* (Muthu et al. 1984).

Ethanedinitrile

Ethanedinitrile (C_2S_2, Cyanogen) was discovered in 1815 (Gay-Lussac 1815). O'Brien et al. (1999) patented ethanedinitrile as a broad-spectrum alternative fumigant to methyl bromide for uses against insects, arachnids, nematodes, bacteria, molds, and rodents in a variety of commodities, including but not limited to grain, seeds, meats, fruit, vegetables, timber, and plants. Brotherton and Lynn (1959) reviewed the physical and chemical properties of ethanedinitrile. Ethanedinitrile has a boiling point of -21 °C with a vapor pressure of 515 kPa at 21 °C (McConville and Swaminathan 2017). The national institute for occupational safety and health (NIOSH) has a recommended exposure limit (REL) of 10 ppm (v/v), which compares favorably to both methyl bromide (5 ppm) and phosphine (0.3 ppm). Another benefit of this compound is that it is not an ozone-depleting substance (Ren et al., 2005). A few studies on ethanedinitrile have shown control against *R. dominica* (F.) (Ren and Trang 2003, Hooper et al. 2003, Ren et al. 2014), *S. granarius* (L.) (Hooper et al. 2003), *S. oryzae* (L.) (Ren and Trang 2003, Hooper et al. 2003, Ren et al. 2014), *T. castaneum* (Herbst) (Ren and Trang 2003, Hooper et al. 2003, Ren et al. 2014), *T. confusum* du Val (Ren and Trang 2003, Hooper et al. 2003), *E. cautella* (Walker) (Hooper et al. 2003), *Trogoderma variable* (Ballion) (Ren et al. 2014) and *L. serricorne* (F.) (Ren et al. 2014).

Life Histories of Target Pests

There is a need for alternative fumigants to be available for integrative pest management programs to control pests and reduce post-harvest losses. As previously mentioned, propylene oxide, ethyl formate, and ethanedinitrile are effective against several stored product pest species. However, ethyl formate, propylene oxide, and ethanedinitrile's toxicity and effectiveness to control *L. serricorne*, *T. putrescentiae*, or *Necrobia rufipes* (De Geer) have not been well-

established. Thus, in order to test if the proposed alternatives could control these pests, it is best to understand each pest's status, biology, and management.

Cigarette Beetle

Lasioderma serricorne (Fabricius, 1792) (Coleoptera: Anobiidae) is known as the cigarette beetle. The cigarette beetle is a cosmopolitan pest of durable post-harvest products, which causes substantial postharvest losses worldwide (Runner 1919; Howe 1957; Hagstrum et al. 2013). Hagstrum et al. (2013a) reported an extensive list of *L. serricorne* infestations on 222 types of dried plant and animal products. The biology of *L. serricorne* has been well studied and reviewed by earlier authors (Runner 1919; Rayner 1951; Howe 1957; Sivik et al. 1957; Ashworth, 1993; Edde 2019). *L. serricorne* takes 29-49 d to complete its life cycle at optimal conditions. Female *L. serricorne* lay eggs within 12 h of mating and on average, oviposit between 40-76 eggs in their lifetime; however, fecundity is dependent on food quality, quantity, and type. The newly emerged larva often consumes their eggshell following emergence and tend to prey on eggs around them. Four larval instars occur before a cocoon for pupation is constructed where the larva lie quiescent for two to four days.

After pupation, the adult stays in the pupal cell for four to six days before emerging to mate. Mating occurs two to three days following emergence with the survival of adults lasting two to seven weeks. Adult *L. serricorne* penetrates packaged commodities by chewing holes to enter the packaging (Athanassiou et al. 2011). The beetles substantially reduce the market value of commodities by reducing the quantity of the product through consumption, and the product quality is reduced by leaving behind dead bodies, cast skins, and frass (Edde et al. 2012). Management strategies to control *L. serricorne* have included sanitation, exclusion (Vinzant and Reed 1941), monitoring traps (Faustini et al. 1990, Papadopoulou and Buchelos 2002), mating

disruption (Mahroof and Phillips 2014), temperature management (Hansen et al. 2011; Yu et al. 2011), controlled atmospheres (Edde 2019), irradiation (Imai et al 2006; Kongratarpon 2003), residual pesticides (Getchell and Subramanyam 2007), and fumigation (Zettler and Keever 1994; Sağlam et al. 2015; Su and Scheffran 1990; Gautam et al. 2014; Ryan et al. 2006).

Mold Mite

Tyrophagus putrescentiae (Schrank) (Sarcoptiformes: Acaridae) is commonly known as the mold mite, the ham mite, the cheese mite, or the copra mite. Biology and management strategies for *T. putrescentiae* have been reviewed by Zhao et al. (2016), and Zhang et al. (2018). *T. putrescentiae* is an associated pest of over 140 commodities (Hagstrum et al. 2013b). *T. putrescentiae* infests many commodities that contain high-protein and high-fat contents, such as dry-cured meats (Rentfrow et al. 2006), artisanal cheeses (Rentfrow et al. 2008, Krishnan et al. 2019), commercial pet food (Thind 2005, Brazis et al. 2008, Rybanska et al. 2016), and dried fruits (Dizlek et al. 2019). *T. putrescentiae* infestations usually occur on the surface of a product; however, penetration of the commodity can occur (Zdárková 1991). Considerable economic damage can occur between infestation, penetration, and the mite's ability to cross-contaminate commodities with aflatoxin producing fungi (Franzolin 1999). Infestations are not readily detectable by sight and commonly persist unnoticed until a "dust" of mites' forms over the surface of the product, as they are of minute size with females ranging from 320–420 µm and males ranging 280-350 µm long. *T. putrescentiae* can complete one generation in 8–53 d depending on the infested commodity, temperature, and relative humidity (Rinkikumari and Shukla 2019). The life cycle progression of *T. putrescentiae* is an egg, a six-legged larva, a protonymph, a tritonymph, and finally an adult (Vacante 2016). A female will begin laying eggs within 24 h of mating and can oviposit up to 500 eggs, which creates a capacity for the

population to grow exponentially (Boczek 1991). The mite's capacity for exponential population growth combined with its minute size and its ability to spread detrimental aflatoxins cause extreme difficulties for facility management strategies. Management strategies such as sanitation, monitoring traps (Thind 2005, Amoah et al. 2016, 2017a), temperature management (Abbar et al. 2016b), light type use (Amoah et al. 2017b), food-safe coatings (Abbar et al. 2015, Zhao et al. 2016, Campbell et al. 2017), food-grade netting (Zhang et al. 2017), residual pesticides (Abbar et al. 2016a), and fumigation (Phillips et al. 2008, Zhao et al. 2015, Abbar et al. 2018) have been tested to eliminate or suppress mite populations. The phase-out of methyl bromide has left many stored product managers to turn to tested and approved methods that suppress mite populations in order to minimize the disruption of their product's market.

Red-legged Ham Beetle

Necrobia rufipes (De Geer, 1775) (Coleoptera: Cleridae) was given the common name of the red-legged ham beetle by C.V. Riley (Simmons and Ellington 1925). *N. rufipes* is a pest of 102 commodities, including dry-cured ham, dried fish, copra, pet food, cheese, and carrion (Hagstrum and Subramanyam 2009). The biology and management strategies of *N. rufipes* were reviewed by Simmons and Ellington (1925), and such a comprehensive review has not been found since that time. A brief summary of *N. rufipes* biology is stated here. *N. rufipes* are voracious facultative predators that cannibalize one another. It takes 30–107 d for *N. rufipes* to complete its life cycle at optimal conditions (Simmons and Ellington 1925, Osuji 1977). Female *N. rufipes* lay eggs in clusters of 2–12 within two days of mating, and they can oviposit 40–900 eggs in their lifetime. However, fecundity is dependent on food quality, quantity, and type (Simmons and Ellington 1925, Bhuiyan and Saifullah 1997, Nalinakumari and Mammen 1998a). The newly emerged larva often consumes their eggshell following emergence and tend to

cannibalize eggs around them. Three to four larval instars occur before a cocoon for pupation is

constructed where the larva lies quiescent for about two weeks. After pupation, the adult stays in

the pupal cell for one to two days before emerging to mate. Mating occurs two to three days

following emergence with the survival of adults lasting two to seven weeks. Adults are metallic

blue-green black and 3.5-4.5mm long. The longevity of an adult is, on average, two months but

can be up to 14 months under optimal conditions. Males and females are sexually dimorphic

with stiff black hairs arising from elytral punctures anteriorly in females and posteriorly in males

(Simmons and Ellington 1925). *N. rufipes* larvae penetrate packaged commodities by chewing

and tunneling holes through commodities. The adult beetles pull dry-cured meats away from the

bones causing moisture reduction of the meat to be nonuniform (Simmons and Ellington 1925).

These beetles ultimately reduce the market value of infested products by lowering the product

and reducing the product available for sale. Management strategies to control *N. rufipes* include

controlled atmospheres (Odeyemi and Akinnusi 1985, Hasan et al. 2016), irradiation (Ahmed et

al. 1989, Alam 2004), botanicals (Ward and Golob 1994, Ahmed et al 2013), contact insecticides

(Walker 1987, Roesli et al. 2003), and fumigation (Mallamaire 1957, Nalinakumari et al. 1998b,

Phillips et al. 2008).

Study Objectives and Justification

Due to the ban of methyl bromide, the corrosive properties of phosphine, the increasing

global populations of *L. serricorne* evolving resistance to phosphine, and the ineffectiveness of

sulfuryl fluoride to control *L. serricorne*, *T. putrescentiae* or *N. rufipes,* there is a necessity for

alternative fumigants to control these pests and reduce post-harvest losses. As previously

mentioned, propylene oxide, ethyl formate, and ethanedinitrile are effective against several

stored product pest species. However, ethyl formate, propylene oxide, and ethanedinitrile's

toxicity and effectiveness to control *L. serricorne*, *T. putrescentiae*, or *N. rufipes* have not been well-established.

Thus, experimentation on *L. serricorne* and *T. putrescentiae* were conducted with propylene oxide or ethyl formate to show that (1) either fumigant added at a series of increased concentrations would increase the target pest's mortality until the target pest mortality reached 100%, (2) either fumigant held at a fixed concentration over a span of varying time (1, 2, 3, 4, 5, 6, and 12 h) would result in a target pest's mortality until the target pest's mortality reached 100%, (3) a concentration of either fumigant estimated to cause ~99% mortality in a specific pest population (4) either fumigant added to different commodities (i.e., flour, tobacco, pet food, fishmeal or dry-cured ham) in a fumigation chamber would impact the mortality of a pest differently as the interactive properties of the experimental gas and the commodity would be different. Experimentation on *N. rufipes* was conducted to confirm that propylene oxide or ethyl formate applications to control ~99% of *T. putrescentiae* populations in a mixed life stage colony would also control ~99% of *N. rufipes* mixed life stage populations. Lastly, experimentation on *T. putrescentiae* with ethanedinitrile was conducted to show that ethanedinitrile at a series of increased concentrations, caused an increase in *T. putrescentiae* mortality until 100% mortality was achieved, and validation of the control efficacy was confirmed using mixed life stage populations of *T. putrescentiae* population.

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Chapter 2 - Efficacy of liquid fumigants propylene oxide and ethyl formate to control *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae)

Introduction

Lasioderma serricorne (F.) (Coleoptera: Anobiidae) is a cosmopolitan pest of durable post-harvest products, which causes substantial postharvest losses worldwide (Runner 1919, Howe 1957, Hagstrum et al. 2013). *L. serricorne* has been reported to infest up to 222 types of dried plant and animal products (Hagstrum et al. 2013). *L. serricorne* biology is well established (Runner 1919; Rayner 1951; Howe 1957; Sivik et al. 1957; Ashworth, 1993; Edde 2019). *L. serricorne* is known to take 29-49 d to complete its life cycle at optimal conditions with females able to lay eggs within 12 h of mating. On average, females oviposit 40-76 eggs in their lifetime; however, fecundity is dependent on food quality, quantity, and available type. Newly emerged larvae consume their eggshells following emergence and tend to prey on eggs in the vicinity. Pupation occurs in a constructed cocoon made with larval spit, feces, and other material after four larval instars. After pupation, the adult stays in the pupal cell for four to six days before emerging to mate. Adults mate in the first three days following emergence and have a longevity of two to seven weeks.

Adult *L. serricorne* enter packaged commodities by chewing holes to leave or enter the product (Athanassiou et al. 2011). This substantially reduces the market value of the commodity by damaging the packaging and the beetles then continue to reduce the quantity and quality of the product by leaving behind dead bodies, cast skins, and frass (Edde et al. 2012). Since *L. serricorne* is a pest of several high-valued durable commodities, many management techniques have been employed to reduce loss, such as sanitation, monitoring, and mitigation. The most popular and effective mitigation technique to date is fumigation (Phillips et al. 2012; Edde 2019). For over 70 years, methyl bromide was used as a broad-spectrum fumigant to control stored

product pests, including *L. serricorne*; however, the international Montreal Protocol treaty required the phase-out of the compound due to its ozone-depleting properties (Oberthür 1997).

Phosphine is currently the most popular method that is used for controlling stored product insects. Phosphine was first used in 1930 to control stored product pests in Germany (Fluck 1973, Heseltine and Thompson 1957), and phosphine began to be used for fumigation of cured tobacco leaves to control *L. serricorne* in the U.S. in 1960. However, evidence for genetically inherited resistance to phosphine in several storage insect pests, including *L. serricorne*, is accumulating in many countries due to heavy use. For example, in 2003, 31 *L. serricorne* populations from 12 countries were resistant to phosphine, and by 2015, all U.S. *L. serricorne* populations that were tested were resistant to phosphine (Savvidou et al. 2003, Sağlam et al. 2015). Additionally, phosphine is highly corrosive to copper, the major conductor used in many electrical wirings, which makes it an undesirable fumigant for some storage or marketing facilities that *L. serricorne* infests (Bond et al. 1984).

Sulfuryl fluoride is a structural fumigant that was registered to control pests in stored products in 2005 as an alternative to methyl bromide and to combat phosphine resistance. Sulfuryl fluoride is not an adequate alternative, as it has not been registered for use on tobacco that *L. serricorne* infests. Residual fluoride ions can exceed thresholds in certain foods, and it can be ineffective for controlling *L. serricorne* eggs (EPA 2005, Su and Scheffrahn 1990). Thus, alternative fumigants are needed so that different compounds can be used in a fumigation rotation in integrated pest management programs for *L. serricorne*.

Alternative fumigants such as propylene oxide (C_3H_6O) (PPO) and ethyl formate ($C_3H_6O_2$) (EF) have approval by government agencies in the U.S. to be applied to commodities; however, not as fumigant pesticides (FDA 2018, Griffith and Warren 2000). PPO is approved as

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a microbial sterilant, and EF is a food flavoring agent approved as a generally recognized as safe compound (Griffith 1999, FDA 2018). PPO is a colorless liquid at room temperature with a boiling point of 34 °C that is flammable at concentrations of 3-37% volume by volume (v/v) in air. The flammability of this compound has been tempered with carbon dioxide, which led to its approval as a microbial sterilant in the U.S. (Jones 1933, Griffith and Warren 2000). PPO has been used as a microbial sterilant since 1958 and was used as an insecticide until 1988 when re-registration lapsed under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) amendment (Griffith 1999, EPA 2016). PPO has insecticidal properties that can control stored product pests (Griffith 1999, Zettler et al. 2003, Isikber et al. 2004, Navarro et al. 2004, Isikber et al. 2006, Gautam et al. 2014). EF is also a colorless liquid at room temperature with a boiling point of 54 °C that is flammable at concentrations of 2.8-16.5% (v/v) in air. In the U.S., EF is labeled as a "generally recognized as safe" (GRAS) compound and food additive by the FDA with an allowance of 0.01%-0.05%, depending on the food item (FDA 2018). EF is useful at controlling stored product insects such as, *Sitophilus oryzae* (Muthu et al. 1984, Damcevski and Annis 2002, Xin et al. 2008), *Tribolium castaneum* (Muthu et al. 1984, Damcevski and Annis 2002, Xin et al. 2008), *Rhyzopertha dominica* (Damcevski and Annis 2002, Xin et al. 2008) and *Sitotroga cerealella* (Muthu et al. 1984). In Australia, a commercial formulation of EF has been approved for the disinfestation of postharvest pests on grains and additional commodities (Ryan et al. 2006). A benefit of PPO and EF is that neither are ozone-depleting substances.

Although many studies indicate that PPO and EF can control stored product pests, studies on these alternatives to control *L. serricorne* are lacking. Therefore, the purpose of this study was to determine the efficacy of PPO and EF by evaluating the concentrations and exposure times at which the most tolerant life stage of *L. serricorne* would reach 100% mortality and

verify these results through mixed life stage fumigations. Lastly, this study used bioassays to determine if sorption, the chemical or physical process of the gas attaching to the commodity, or desorption, residual gas released from the commodity after a fumigation's venting period, would impact mortality or survival of the pest on flour, a commercial pet food, and flue-cured tobacco.

Materials and Methods

Beetle Culture and Life Stage Separation

Laboratory cultures of *L. serricorne* were established with insects collected near Tifton, GA in 2014. Beetles were reared on Heartland Mills® organic whole golden buffalo wheat flour diet containing 5% brewer's yeast by weight in 1L glass wide-mouthed Ball® Mason jars with 9 cm diameter metal mesh screened lid. Colonies were maintained in a growth chamber at 27 ± 3 °C with 70% R.H. and a 16:8 L:D photoperiod.

General insect rearing and separation methods followed Sağlam et al. (2015). Adult beetles were separated from 4-week-old cultures using vacuum tweezers (Tweezer-vac™, Vacuum System TV-100 Virtual Industries, Inc.) following sifting with a U.S. No. 20 sieve. The adults were placed in 11 mL glass vials containing 0.5 g of diet that was secured with a fabric mesh and a ventilated plastic push-on lid. Vials were held at the same rearing conditions for up to 6 h before fumigation. Larvae were extracted from 2-week-old cultures using the same techniques that were used to separate adults. Pupae were isolated by first sifting large larvae from 3-week-old cultures and placing them on 1 g of rearing diet followed by holding at the same conditions for 96 h to allow adequate time for larvae to complete development into pupae. Eggs were obtained by first isolating 200 unsexed adults from 4-week-old cultures and placing them in 118 mL glass jars with 1 g of organic wheat flour that was triple-sifted with a U.S. No. 120 sieve and secured with a 7 cm diameter metal screen and ring cap. Adults were left to mate

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and lay eggs for 24 h in the growth chamber. A U.S. No. 20 and U.S. No. 80 sieve were used to separate the adults and eggs, respectively, from the oviposition flour. Eggs were then individually extracted using a five-bristled golden Taklon® paintbrush and placed in 4 mm diameter wells that were formed in a plastic slide. The welled plastic slide was covered with a glass slide and secured with 0.9 x 1.5 cm steel binder clips and kept under standard rearing conditions for up to 3 h prior to fumigation (Figure 2.1).

Efficacy Bioassays

Fumigant Concentration-Mortality Assays

Propylene oxide and ethyl formate were purchased from Sigma Aldrich (Milwaukee, WI, USA) at purities of ≥ 99% and 97%, respectively. One-liter glass wide-mouthed jars were loaded with vials of adults, pupae, larvae, or eggs and an 11 mL glass vial with 2 mL of water. The 11 mL glass vial containing 2 mL of water was used to maintain humidity. The iced undiluted liquid toxicant volumes were applied into each jar with either a calibrated 0.5–10 µL (Molecular Technologies, AlphaαPette), 2–20 µL, or 20–200 µL micropipette (Rainin, Classic). Toxicant volumes were converted from microliters to a toxicant concentration milligrams per liter of toxicant applied based on the volume of the jar that would be used in fumigation and the density of the liquid fumigant used. Toxicant volumes were applied along the internal sidewall of each glass jar inside a fume hood. Jars were immediately sealed and secured with a gas-tight metal canning lid and screw ring. Jars were placed in an incubator set at 25 ± 1 °C with 70% R.H. and a 16 h photoperiod for 24 h. After 24 h, jars were opened and ventilated in the fume hood for 10 min. Vials were then removed from fumigation jars and placed into an incubator at 29 ± 1 °C with 70% R.H. 16:8 L:D for a recovery period. The recovery periods for PPO experiments were 72 h for adults, 96 h for pupae, 168 h for larvae, and 192 h for eggs and the

recovery periods for EF experiments were 72 h for adults, 144 h for pupae, 96 h for larvae, and 192 h for eggs. Adult mortality was determined by the inability to move following a physical stimulation with a wooden-handled dissecting needle (Carolina Biological Supply Company, Burlington, NC), and eggs were scored as dead by failing to hatch to first instar within 7 d of treatment. Other immature stages were scored as dead by the inability to progress to the next life stage in the given time period. Any mobile stage that did not physically react following stimulation with a dissecting probe at the end of a life stages' set recovery period were scored as dead. Fumigant concentration-response assays were replicated 2–8 times per concentration.

Time-Mortality Assays

Experiments on the amount of time needed to achieve a given level of mortality were conducted with methods that were described in the fumigant concentration-mortality assays to determine the most tolerant life stage from the concentration-assays. Specimens were tested at the concentration that consistently caused 100% mortality of the most tolerant life stage in 24 h, and the fumigant exposure time varied at periods of 1, 2, 3, 4, 5, 6, 9, and 12 h. Time response assays were conducted in duplicate.

Verification of Fumigation Efficacy in Mixed Life Stage Colonies

The ability of the fumigants to kill individuals in a small population was evaluated in fumigations against mixed life stage colonies of *L. serricorne* using methods described by Sağlam et al. (2015). Fifty adults from 4-week-old cultures were isolated using vacuum tweezers and a U.S. No. 20 sieve into a 118 mL glass jar containing 20 g of organic flour and 1 g of Brewer's yeast to create mixed life stage cultures. These cultures were then placed into an incubator at 29 ± 1 °C with 70% R.H. 16:8 L:D. An additional 50 adults were added to each culture one week later to create overlapping generations. All adults were removed with vacuum

tweezers three weeks from the original infestation date, and colonies were left to develop in an incubator set at 29 ± 1 °C with 70% R.H. 16:8 L:D for two weeks. Mixed life stage colonies were loaded into a 3.8 L glass jar with a 118 mL glass jar containing 8 mL of water. The 118 mL glass jar containing 8 mL of water was added to maintain humidity. Toxicant volumes calculated to concentrations of 100, 500, and 1000 mg/L, which was 1, 5, and 10x of the concentration that consistently caused 100% mortality in the initial concentration-mortality studies, were applied to each fumigation jar with a 200–1000 µL micropipette. The liquid fumigant was applied along the inner side of each glass jar inside a fume hood as before. Jars were sealed and secured with a metal canning lid and canning ring. Jars were placed in an incubator that was set to 25 ± 1 °C and 70% R.H. with a 16 h photoperiod for 24 h. After 24 h, jars were vented in the fume hood for 10 min. Mixed life stage colonies were removed from fumigation jars and placed into an incubator at 29 ± 1 °C and 70% R.H. 16:8 L:D for a recovery period of 72 h. Mixed life stage colonies were sieved with a U.S. No. 20 sieve. Adults were scored by determining their mortality following their recovery period and placed back into the incubator. Fumigated mixed life stage colonies were sieved, and emerged adults were scored every week for six weeks following the fumigation. Mixed life stage fumigations were conducted in replicates of five.

Sorption and Desorption Bioassays

Mortality assays were conducted to determine if fumigant sorption by a commodity could affect the toxicity of an applied fumigant. Sorption bioassays were conducted in 3.8 L glass wide-mouthed jars containing 200 g of one of the three commodities: organic golden buffalo wheat flour (Heartland Mills, Modoc, KS), commercial mixed dry kibble dog food consisting of a minimum of 23% crude protein, 12% crude fat and 4% crude fiber at a 14% moisture content,

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or flue-cured tobacco (provided by Altria Client Services, Inc., Richmond, VA). Two 11 mL glass vials with 1 g of rearing diet each contained 20 larvae in the PPO assays and 20 pupae in the EF assays. Each vial was secured with a fabric mesh and a ventilated plastic push-on lid and placed above and below the commodity. An opened glass vial containing 8 mL of water was also placed resting on the commodity. Bioassays were treated by adding volumes calculated to equal 70 mg/L of PPO or 100 mg/L of EF to a 9 cm diameter glass Petri dish bottom. Jars were immediately sealed and secured with a gas-tight septum lid and placed in an incubator that was set at 25 ± 1 °C, 70% R.H., and 16:8 (L:D) for 12 and 24 h. After 24 h, jars were ventilated for 10 min. Vials were then removed from fumigation jars and placed into an incubator that was maintained at 29 ± 1 °C, 70% R.H., and 16:8 L:D for a 96 h recovery period. Desorption bioassays were conducted in the previously fumigated jars containing the treated commodities of the sorption experiments to determine if there were levels of toxic gas remaining that would result in pest mortality. Fresh untreated *L. serricorne* pupal vials were added to the jars to replace the previously fumigated vials. The jars were sealed and secured with a gas-tight septum lid and were placed in an incubator set at conditions previously described. Mortality was accessed as described previously in the efficacy bioassay methods. Sorption and desorption bioassays were replicated three times.

Data Analysis

Fumigant concentration-mortality assays and time response assays were analyzed using probit regression in SAS Studio® software (SAS 2018). Sorption and desorption mortality assay data for proportion killed were transformed with an angular transformation and tested for normality and individually analyzed using one-way ANOVA with general linear mixed models

(GLMMIX) (SAS 2018). Means were separated by Tukey's HSD (Honestly Significant Difference) posthoc test at $P<0.05$.

Results

Fumigant Concentration Response Assays

The Pearson goodness-of-fit (χ^2) test showed that the probit regression model's fit to the observed data was significant (P <0.05) for all probit model regressions. Since the calculated P-values of the tests were low, variances and covariances for each test were adjusted by a heterogeneity factor, which is calculated by the Chi-square value (χ^2) divided by the degrees of freedom (df). The critical value from the t-distribution was then used to compute the fiducial limits for the LC_{50} and LC_{99} (SAS 2018).

Eggs were very susceptible to both PPO and EF, such that four concentrations could not be placed into a probit analysis to achieve a projected model. Eggs were treated with PPO at concentrations of 0.93, 1.87, 2.80, 3.74, 4.67, 5.60, 6.54, 7.48, 8.41, 9.35, 11.68, 14.02, 18.69, 32.71, 46.73, and 70.09 mg/L. The control had 363 eggs tested, resulting in an average mortality of 11.57±1.49%. A total of 3402 eggs were tested with the concentrations listed and resulted in 100% mortality for all concentrations except for the eggs treated with 4.67 mg/L. The eggs treated with 4.67 mg/L resulted in an average mortality of 93.83±6.17%. Eggs were treated with ethyl formate at concentrations of 0.00, 0.48, 0.95, 1.43, 1.90, 2.86, 3.81, 4.76, 9.52, 14.28, 19.04, 23.81, 47.61,71.42, 95.22 mg/L. The control had 327 eggs tested, resulting in an average mortality of 9.66±3.46%. A total of 1492 eggs were tested for additional concentrations. Concentrations 0.48, 0.95, and 1.43 mg/L resulted in the average mortality of 93.75±3.89%, 74.37±10.15%, and 91.88±6.49%, respectively. The remaining concentrations that were tested resulted in 100% mortality. The LC_{50} indicated that larvae are the most tolerant life stage to PPO

and pupae are the most tolerant life stage to EF (Tables 2.1, 2.2). The average percent of mortality is graphed against the achieved average concentrations for selected target concentrations (Figure 2.2, 2.3).

Time Mortality Results

Fumigations of PPO at a concentration of 70.09 mg/L against larvae achieved 100% mortality in 3 h (Table 2.3). The PPO fumigations had a sample size of ~80 larvae per time period tested with each replicate having a control. Fumigations of EF at 95.22 mg/L against pupae also achieved 100% mortality in 3 h (Table 2.4). The fumigations had a sample size of ~80 pupae per time period tested with each replicate having a control.

Fumigation Efficacy in Mixed Life Stage Colonies Verification Results

No adults emerged within six weeks following fumigation of either fumigant at concentrations of 100, 500, or 1000 mg/L (Table 2.5).

Sorption and Desorption Results

The sorption of PPO caused significantly different mortalities on cigarette beetle larvae in the commodities for the 12 h exposure time ($F=45.81$, df=3, $P=<0.0001$) and the 24 h exposure time ($F=15.50$, df=3, $P=0.0011$) (Figure 2.4). The sorption of EF did not cause significantly different mortality of pupae in any of the commodities for the 12 h exposure time ($F=1.14$, df=3, $P=0.3915$), but did cause significantly different mortality in the 24 h exposure time ($F=6.76$, df=3, $P=0.0139$) (Figure 2.4). The desorption of either gas did not cause a significant difference in the mortality of the 966 larvae tested nor the 967 pupae tested during either exposure time.

Discussion

Results indicate that the order of *L. serricorne* tolerance to the exposure of PPO is larvae> pupae> adults> eggs. Although an LC_{50} was not determined for eggs in this study, the

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complete mortality of the egg stage was achieved within 24 h using PPO at a concentration of 5 mg/L or less. This observation is similar to a previous study in which the LC_{50} that was needed to achieve complete mortality of *L. serricorne* was an LC_{50} of 2.97 mg/L PPO (Gautam et al., 2014). PPO concentration-mortality data from the current study did not fit the probit model, which may be due to variation in gas dispersal since the gas was held at normal atmospheric pressure and a temperature that was lower than its boiling point (Bond 1989). However, studies conducted at low atmospheric pressures and temperatures that kept PPO in the gas phase have also failed to produce data that fits a probit model (Isikber et al. 2004, Isikber et al. 2006). Although the study showed that cigarette beetle larvae might be controlled within three hours of exposure to propylene oxide, this would not be recommended. Zettler et al. (2003), reported that PPO mixed life stage fumigations of *L. serricorne* reached 100% mortality when exposed for 48 h at 45 mg/L, this was conducted at an 8: 92% (v/v) ratio of PPO to carbon dioxide (CO_2) at normal atmospheric pressure. Zettler et al. (2003) chose this concentration and temperature to test because it is in the standard sterilization protocol of PPO; however, they extended the exposure time to 48 h rather than the 4 h recommended in the PPO sterilization protocol. In contrast, nearly pure liquid propylene oxide was applied directly to fumigation chambers in the current study and allowed the fumigant to vaporize over 24 h at conditions stated in the methods. Therefore, this study achieved 100% mortality of mixed life stages at a higher application rate, but with half the exposure time of Zettler et al. (2003). Mixed life stage fumigations of *L. serricorne* were monitored for a generation following the exposed life stages, which verified that the determined application rate and exposure time of propylene oxide was effective at controlling *L. serricorne*. Zettler et al. (2003), and Isikber et al. (2005, 2006), have quantitatively shown that PPO has high sorption rates in commodities. For example, Zettler et al. (2003) showed high

rates of PPO sorption, with a 95% reduction in raisins, a 93% reduction in figs, and a 78% reduction in walnuts. Isikber et al. (2005, 2006) reported a reduction of the initial gas by 87% in peanuts, 91% in walnuts, 40% in corn, 76% in cocoa beans, 25% in narcissus bulbs, and 41% in wheat. Although PPO sorption was not quantitatively measured in the selected commodities, fumigation results showed that different commodities have different sorption qualities, which can impact pest mortality through presumably drawing the fumigant out of the headspace. This has also occurred in PPO treated *Plodia interpunctella* (Hübner) in peanuts and walnuts (Isikber et al. 2005).

The order of *L. serricorne* tolerance to the exposure of EF by life stage is pupae> larvae> adults> eggs. Two other stored product beetles, *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst), exhibit similar levels of susceptibility stages to EF (Muthu et al. 1984). Damceviski and Annis (2002) reported that EF at an application rate of 90 g/m^3 (90 mg/L) with a 24 h exposure could control all life stages of *S. oryzae*, *T. castaneum*, and *Rhyzopertha dominica* (F.). All life stages of *L. serricorne* are controllable with an application of EF at a rate of 100 mg/L in 24 h with the most tolerant life stage, pupae, controlled within 3 h of a 95.22 mg/L application. As with PPO, the EF concentration-mortality data did not fit the probit model; this may also be attributed to variation in gas dispersal due to treatment at normal atmospheric pressure at a temperature lower than the boiling point of EF, which could create microhabitats of concentrated gas. Due to this concern, *L. serricorne* emergence was monitored for at least two generations over six weeks to verify that the determined application rate and exposure time of EF was effective for control. Darby et al. (2009) have quantitatively shown that EF has sorption properties in wheat. Although EF sorption was not quantitatively measured in the selected commodities, fumigation results showed that the sorption quality of EF is not as varied across the

tested commodities as was PPO, and enough exposure of EF toxicity was achieved to result in pest mortality in the presence of the commodity.

This study shows that liquid fumigant alternatives such as PPO and EF are effective at controlling *L. serricorne* on selected commodities and that sorption of PPO and EF are independent of application rate. Future studies using commercially available products should be conducted to determine if they are also able to control *L. serricorne* with quantitative sorption and desorption assays on selected products, and the addition of residual analyses. These proposed studies would be an applicable approach to further support these liquid fumigants in receiving regulatory approval from government agencies as fumigant pesticides on more commodities in the U.S.

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Table 2.1 The Probit analyses of PPO toxicity against *L. serricorne* life stages exposed to fixed concentrations during 24 h exposures at 25°C.

Life Stage	n	Slope±SE	Intercept±SE	LC_{50} (F.L.) (mg/L)	LC_{99} (F.L.) (mg/L)	χ^2	DF	$P > \chi^{2a}$	R^2
Larva	1103	2.44±0.51	-3.93±0.76	41.08 (31.89–60.84)	369.41 (169.18–2494)	225. 85	26	<0.01	0.58
Pupa	1078	1.40±0.10	-1.24±0.13	16.04 (15.52–18.42)	43.88 (36.96–55.85)	89.98	26	<0.01	0.89

a The *P*-value smaller than 0.05 indicates a lack of statistically significant fit between the observed data and the expected regression line.

Table 2.2 The Probit analyses of EF toxicity against *L. serricorne* life stages exposed to fixed concentrations during a 24 h exposure at 25°C.

Life Stage	n	Slope±SE	Intercept±SE	LC_{50} (F.L.) (mg/L)	LC_{99} (F.L.) (mg/L)	χ^2	DF	$P > \chi^{2a}$	R^2
Larva	1111	8.38±1.85	-9.90±2.25	15.22 (13.36–16.75)	28.84 (23.70–47.38)	357.05	26	<0.01	0.53
Pupa	1958	2.67±0.34	-3.87±0.44	28.14 (21.68–39.27)	209.68 (119.65–521.62)	138.67	46	<0.01	0.61
Adult	674	2.68±0.71	-0.6742±0.24	1.78 (1.24–2.49)	13.12 (6.20–186.64)	147.81	14.00	<0.01	0.54

a The *P*-value smaller than 0.05 indicates a lack of statistically significant fit between the observed data and the expected regression line.

Table 2.3 The mortality of *L. serricorne* larvae (n=2) following various exposure times to given concentrations of PPO at 25 ºC.

Time (h)	Average Percent Mortality±S.E.
	PPO treated Larvae at 70.09 mg/L
0	0.0±0.0
1	21.4±4.3
2	90.0±10.0
3	100.0±0.0
4	100.0±0.0
5	100.0±0.0
6	*
9	*
12	*

*Experimentation was not conducted

Table 2.4 The mortality of *L. serricorne* pupae (n=2) following various exposure times to given concentrations of EF at 25 ºC.

Time (h)	Average Percent Mortality±S.E.
	EF treated Pupae at 95.22 mg/L
0	0.23±0.23
1	91.7±3.3
2	93.5±4.0
3	100.0±0.0
4	100.0±0.0
5	100.0±0.0
6	100.0±0.0
9	100.0±0.0
12	100.0±0.0

Table 2.5 Emergence of adults at given days post fumigation of either PPO or EF on *L. serricorne* mixed life stage fumigations at concentrations 1, 5, and 10x the concentrations that cause 100% mortality.

Recovery Days	Emergence at Different Concentrations (mg/L)*			
	PPO		EF	
	0 mg/L	100 mg/L	0 mg/L	100 mg/L
3	2162.0±141.7	0	1380.6±190.6	0
7	1641.0±328.2	0	72.6±18.5	0
14	100.2±30.5	0	5.6±3.1	0
21	6.2±1.4	0	1.0±0.6	0
28	0.2±0.2	0	20.4±5.0	0
35	304.0±56.7	0	444.4±99.5	0
42	1373.6±188.0	0	1503.4±121.5	0

*Experiments testing concentrations 500 and 1000 mg/L also resulted in no adults emerging for the full 42 d.
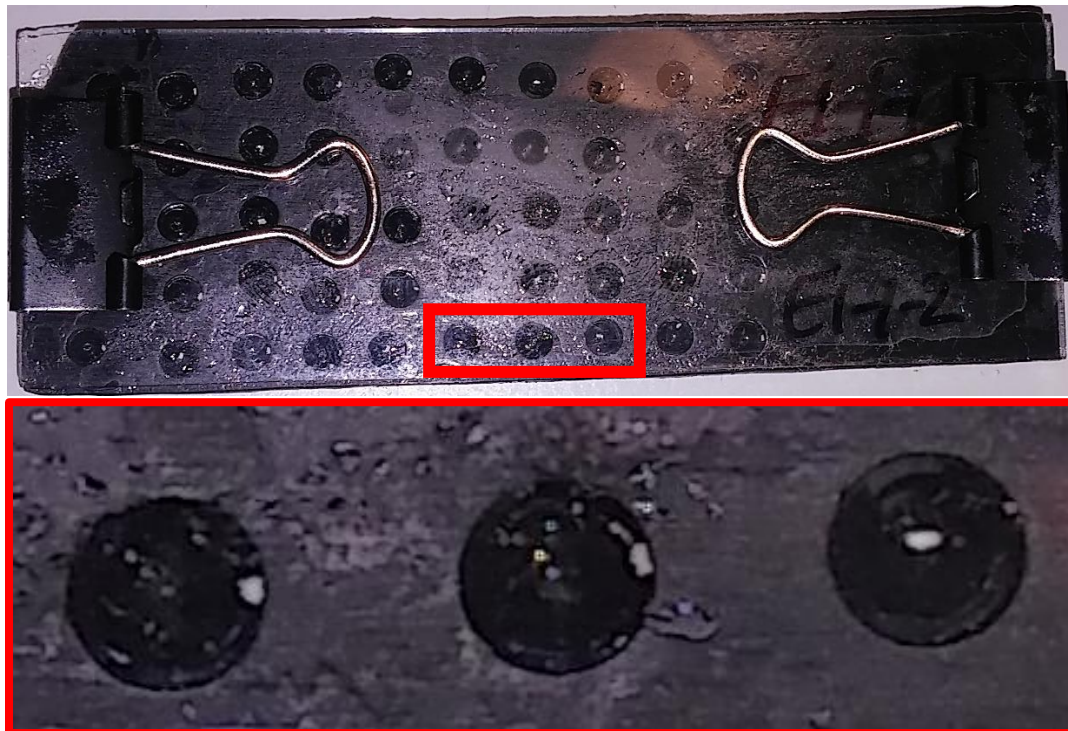
Figure 2.1 The top image is a top-down view of the plastic welled slide that is covered with a standard glass microscope slide and secured with metal bulldog clips. The bottom image is of the individual eggs in their wells at 30x magnification.
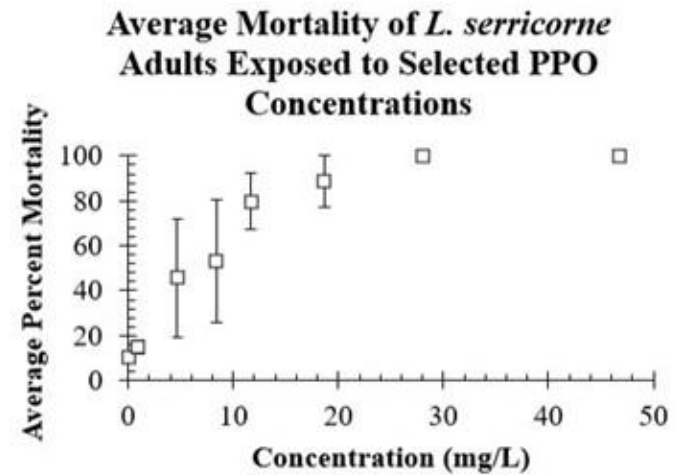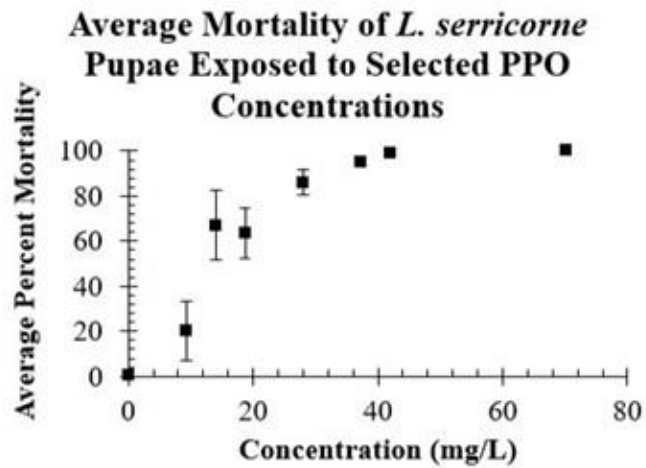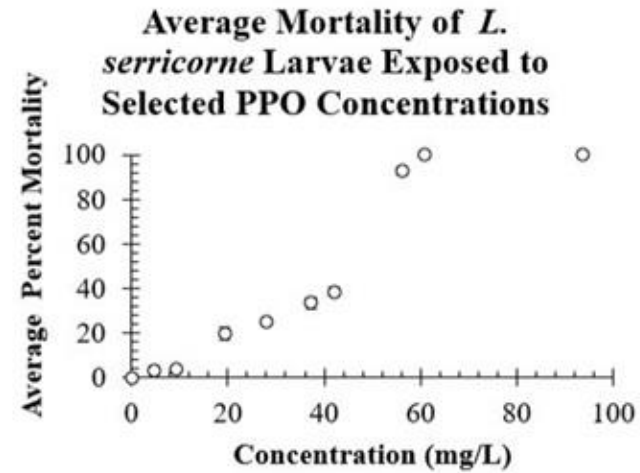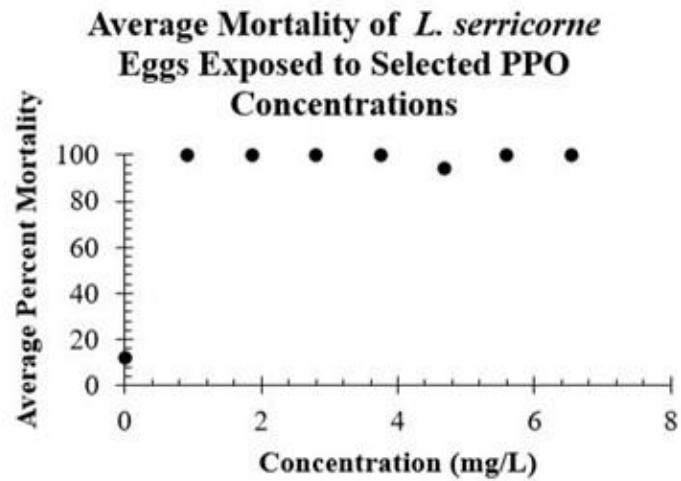
Figure 2.2 The PPO concentration-dependent mortality of *L. serricorne* life stages exposed for 24 h at 25 °C. Each treatment was replicated 2–8 times (n=2~8).
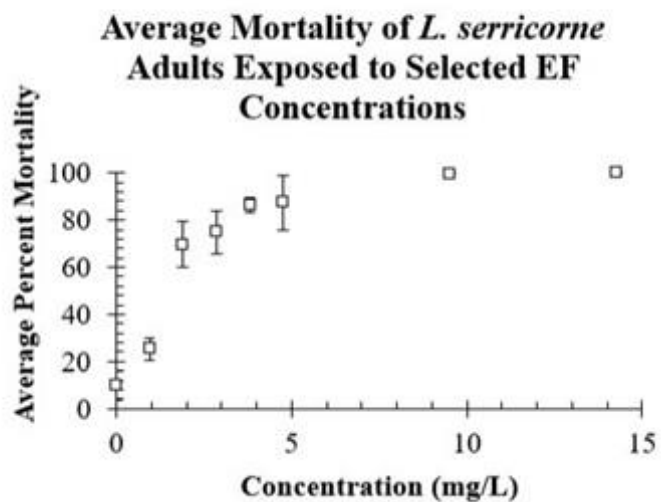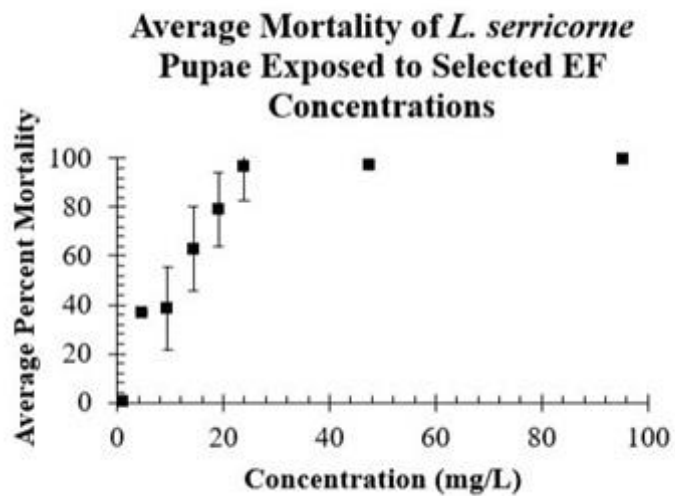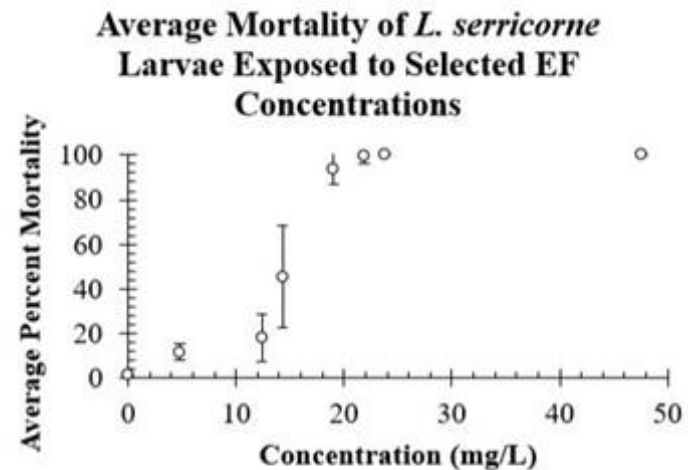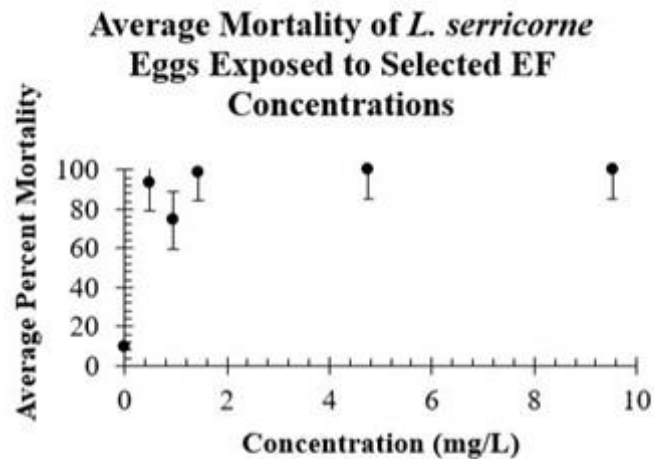
Figure 2.3 The selected EF concentration-dependent mortality of *L. serricorne* life stages exposed for 24 h at 25 °C. Each treatment was replicated 2–8 times (n=2~8).
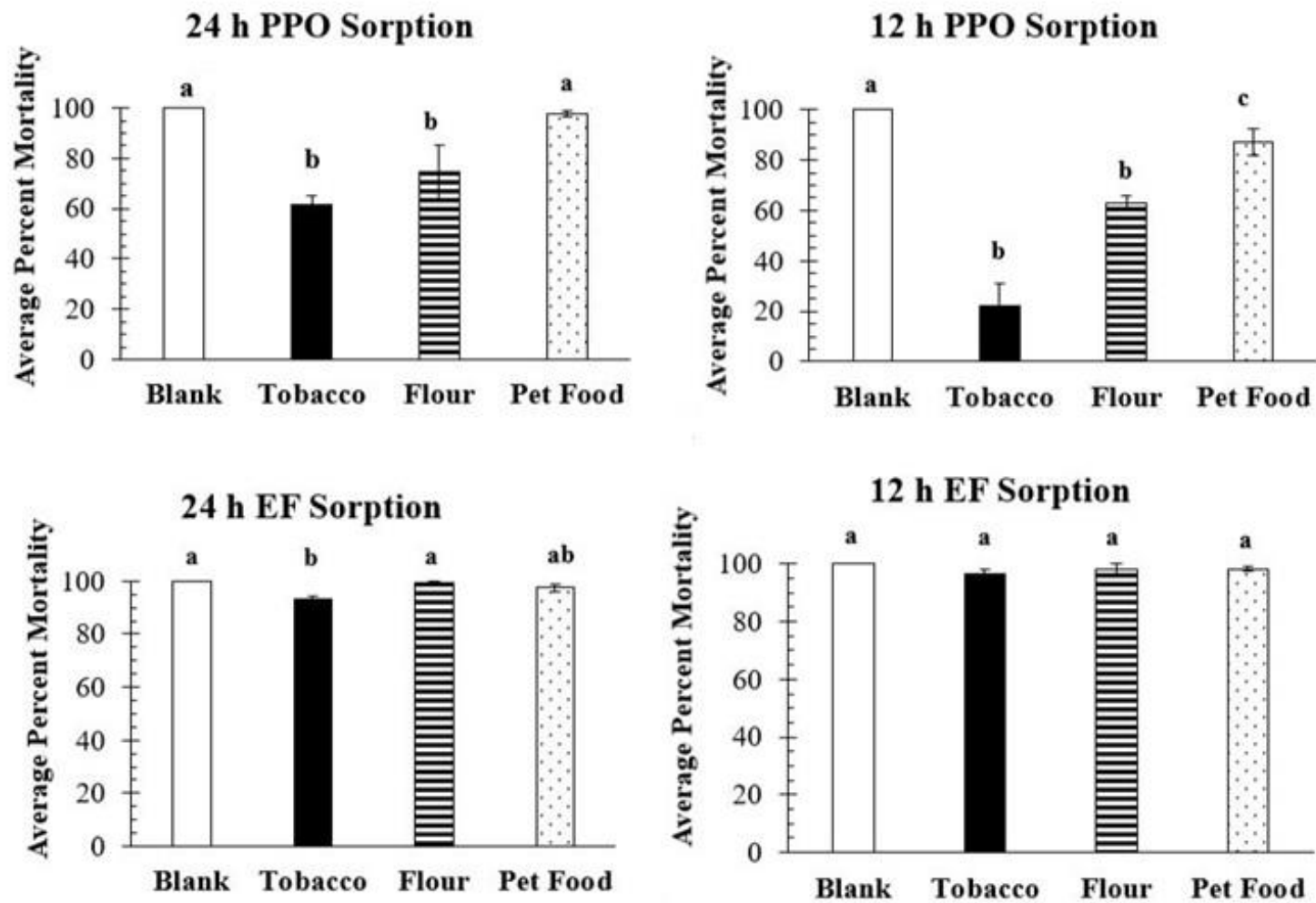
Figure 2.4 Sorption bioassays of average percent mortality and standard errors on three commodities: U.S. flue-cured tobacco, flour, and commercial pet food. The top two figures are PPO larvae bioassays that were conducted for 24 h (n=510) and 12 h (n=491) at 100 mg/L, and the bottom two figures are EF pupae bioassays that were conducted for 24 h (n=468) and 12 h (n=480) at 100 mg/L.

Chapter 3 - Efficacy of Ethyl Formate and Propylene Oxide to Control Ham Pests

Introduction

Tyrophagus putrescentiae (Schrank, 1781) (Sarcoptiformes: Acaridae) and *Necrobia rufipes* (DeGeer) (Coleoptera: Cleridae) known as the mold mite and the red-legged ham beetle are pests of more than 100 commodities (Hagstrum et al. 2013, Hagstrum and Subramanyam 2009). Both are pests of high-value products, containing high protein and fat concentrations, such as dry-cured meats, artisanal cheeses, commercial pet food, dried fruits, and dried fish (Simmons and Ellington 1925, Krishnan et al. 2019, Brazis et al. 2008, Dizlek et al. 2019, Hasan and Phillips 2010).

T. putrescentiae infestations cause a considerable amount of economic damage between infestation, penetration (Zdárková 1991), and the mite's ability to cross-contaminate commodities with aflatoxin producing fungal spores (Franzolin 1999). The biology and management strategies of *T. putrescentiae* have been reviewed by Zhao et al. (2016), and Zhang et al. (2018). *T. putrescentiae* can complete one generation in 8–53 d, depending on the infested commodity, temperature, and relative humidity (Rinkikumari and Shukla 2019). The life stage progression of *T. putrescentiae* is an egg, a six-legged larva, a protonymph, a tritonymph, and finally an adult (Vacante 2016). Adult females are 320–420 µm long, and males range from 280–350 µm long. A female will begin laying eggs within 24 h of mating and can oviposit up to 500 eggs, which allows the population to grow exponentially (Boczek 1991).

N. rufipes infestations initiate with adults locating a viable food source and ovipositing on it (Walker 1987); then, *N. rufipes* larvae penetrate packaged commodities by chewing holes to enter the commodities and tunnel through it. The beetles ultimately reduce the market value of products by reducing the quantity of the product available through consumption and reducing the quality by leaving behind dead bodies, cast skins, and frass. The biology and management of *N.*

rufipes has been studied and reviewed by Simmons and Ellington (1925). *N. rufipes* take 30–107 d to complete a life cycle at optimal conditions (Simmons and Ellington 1925, Osuji 1977). Female *N. rufipes* lay eggs in clusters of 2–12 within two days of mating after which they can oviposit between 40–900 eggs in their lifetime; however, fecundity is dependent on the food quality, quantity, and type (Simmons and Ellington 1925, Bhuiyan and Saifullah 1997, Nalinakumari and Mammen 1998). The newly emerged larva often consumes its eggshell and tends to cannibalize eggs in its vicinity. Three to four larval instars occur before a cocoon for pupation is constructed where the larva lies quiescent for about two weeks. After pupation, the adult stays in the pupal cell for one to two days before emerging to mate. Mating occurs two to three days following emergence with the survival of adults lasting seventeen to sixty-one weeks. Adults are metallic blue-green black, and 3.5–4.5mm long (Simmons and Ellington 1925).

Methyl bromide fumigations were used to control stored product pests for over 70 years until it was discovered that methyl bromide has ozone-depleting properties. In the 1990s, the Montreal Protocol treaty was signed by countries at the United Nations to phase-out methyl bromide and other ozone-depleting substances (Goupil 1932, Oberthür 1997). Since the ban of methyl bromide, several management strategies to control *T. putrescentiae* or *N. rufipes* have been tested in the effort to reduce economic losses, these strategies include monitoring traps (Thind 2005, Amoah et al. 2016, 2017a), temperature management (Abbar et al. 2016b), facility light type use (Amoah et al. 2017b), controlled atmospheres (Odeyemi and Akinnusi 1985, Hasan et al. 2016), food-safe coatings (Abbar et al. 2015, Zhao et al. 2016, Campbell et al. 2017), food-grade netting (Zhang et al. 2017), and contact or residual insecticides (Walker 1987, Roesli et al. 2003, Abbar et al. 2016a). Though this research is being conducted, none of these practices have proven as effective as methyl bromide fumigation. Phosphine fumigation is

effective at controlling *T. putrescentiae* and *N. rufipes*; however, its corrosive properties make it inapplicable for many storage facilities that both pests infest (Bond et al. 1984), including dry-dured ha processors (Zhao et al., 2015). Sulfuryl fluoride is also an inadequate alternative, as it is ineffective in controlling *T. putrescentiae* eggs, and its use is limited due to the fluoride anion residue threshold acceptable on commodities (Phillips et al. 2008, EPA 2018). In the U.S., there is a zero-tolerance for the mite in dry-cured pork (EPA 2011). Therefore, alternative fumigants are needed for rotational application use in integrated pest management programs that target *T. putrescentiae* and *N. rufipes* to reduce economic loss to high-valued commodities.

PPO has been used as a microbial sterilant since 1958 and was used as an insecticide until 1988 when re-registration under the FIFRA amendment lapsed (Griffith 1999, EPA 2016). PPO is a colorless liquid at room temperature with a boiling point of 34 °C that is flammable at concentrations of 3–37% volume by volume (v/v) in air. The flammability of this compound has been tempered with carbon dioxide (Jones 1933; Griffith and Warren 2000). In the U.S., EF is "generally recognized as safe" (GRAS) compound and food additive by the FDA with an allowance of 0.01–0.05% depending on the food item (FDA 2018). EF is also a colorless liquid at room temperature with a boiling point of 54 °C and is flammable at levels of 2.8–16.5% (v/v) air. In Australia, a commercial formulation of EF was approved for the disinfestation of postharvest pests on grains and additional commodities (Ryan et al. 2006). Both PPO and EF have insecticidal properties that can control stored product pests (Muthu et al. 1984, Griffith 1999, Damcevski and Annis 2002, Zettler et al. 2003, Isikber et al. 2004, Navarro et al. 2004, Isikber et al. 2006, Xin et al. 2008, Gautam et al. 2014).

Both PPO and EF have been used to control stored product pests, but studies on the use of these alternatives to control *T. putrescentiae* and *N. rufipes* are lacking. Therefore, the

purpose of this study was to determine the efficacy of PPO and EF at controlling *T. putrescentiae* by determining the concentration and exposure time at which the most tolerant life stage would reach 100% mortality and verify these results through mixed life stage fumigations. Mixed life stage verifications of *N. rufipes* were conducted at the concentration determined to cause 100% mortality of *T. putrescentiae*, because *T. putrescentiae* is traditionally more tolerant to fumigants than *N. rufipes* (Zhao et al. 2016). Lastly, bioassays were used to determine if sorption or desorption of the commodities would impact the mortality of *T. putrescentiae* in treated dry-cured ham, commercial pet food, and fishmeal.

Materials and Methods

Ham Pest Cultures

Tyrophagus putrescentiae and *N. rufipes* laboratory cultures have been maintained in the Department of Entomology at Kansas State University since 2008 from active infestations. Mite rearing generally followed methods described by Abbar et al. (2016a). Mite cultures were reared in 1L glass wide-mouthed Ball© Mason jars that contained mite diet and were sealed with a labeled P8 Fisherbrand 9 cm diameter filter paper and the metal lid ring. The mite diet was comprised of 75 g of commercial pet food (a minimum of 23% crude protein, 12% crude fat, 4% crude fiber, and 14% moisture content) and 475 mL water that were combined and heated for 6 min. in a General Electric Co. 0.95 KW Microwave. The heated mixture was then blended until it was smooth in a 120 V Hamilton Beach Blender©. The mixture was spooned into a 1 L beaker where agar, alphacel, yeast, and vitamin mix at a (5:5:5:5) g ratio was added in addition to 25 mL of glycerol, and 5 mL of 15% methyl-P-hydroxybenzoate in 95% ethanol. The cooked mixture was divided evenly and combined with 14 g of commercial pet food in each mite rearing jar. Mites were introduced from a previous healthy culture after the diets cooled to 25 °C. The

mite cultures were then placed in a rearing cabinet at 22.5 ± 2.5 °C and $70 \pm 2\%$ R.H. in darkness.

Necrobia rufipes were reared according to the methods described by Hasan and Phillips (2010). A 1:1:1 ratio of dried fish, whole, and ground commercial pet food (a minimum of 23% crude protein, 12% crude fat and 4% crude fiber at a 14% moisture content), was placed in the bottom of a 560 cm^2 plastic box with three 9 cm diameter folded filter paper refuges. Colony containers were allowed to develop at 29 °C $\pm$ 1 °C with 70% R.H. Each week, dead adults, live adults, and the filter paper refuges were removed from the plastic colony containers. Live adults were placed into newly set-up colony containers at the same conditions.

Mite Life Stage Separation

Healthy protonymphs, deuteronymphs, tritonymphs, and adults were classified as mobiles and selected using a one bristled Talkon® bristle paintbrush from the culture's mass of mites located on the underside of the filter paper lid. The majority of the mites selected were gravid adult females. Twenty selected mites were individually transferred into a 1.8 mL glass vial containing a 3mm^3 piece of country ham. The vials were labeled and covered with 30μm mesh (Fisher Scientific), and a plastic ventilated lid. Vials were stored at 22.5 ± 2.5 °C and $70 \pm 2\%$ R.H. for up to 24 h prior to fumigation.

Eggs were obtained by selecting 50 healthy adults, mainly gravid females, from the culture's filter paper lid. The adults were placed in a 118 mL glass jar with 7.5 g of cooked mite diet mixture and covered with a labeled P5 Fisherbrand 7 cm diameter filter paper and a metal ring. The adults were left for 48 h in a double water bath (22.5 ± 2.5 °C and $70\pm 2\%$ R.H.) in total darkness. Mite eggs were then individually extracted using a one bristled Talkon® bristle paintbrush and placed on double-sided sticky tape that was attached to a piece of black

construction paper that contained a 3 mm^3 piece of ham. Ten eggs on egg sheets were placed into 1.8 mL vials that were covered with 30 μm mite mesh (Fisher Scientific), and a plastic ventilated shelf vial lid. Vials were stored at 22.5 ± 2.5 °C and 70 ± 2% R.H. for up to 3 h until fumigation.

Efficacy Bioassays

Fumigant Concentration-Mortality Assays

PPO and EF were purchased from Sigma Aldrich (Milwaukee, WI, USA) at purities of ≥ 99% and 97%, respectively. One-liter glass wide-mouthed jars were loaded with 1.8 mL vials of 3 mm^3 pieces of 6-month aged commercially available country ham (Brownsville, Tennessee, USA) containing 20 mobiles or 10 eggs per sheet, and 11 mL glass vial with 2 mL of water. Iced undiluted liquid toxicant volumes calculated to fixed concentrations were applied to each jar with a calibrated 0.5–10 μL (Molecular Technologies, AlphaαPette), 2–20 μL, and 20–200 μL micropipette (Rainin, Classic). Toxicant concentrations were calculated from microliters to milligrams per liter of toxicant based on the volume of the jar that would be used in fumigation and the density of the liquid fumigant. Toxicant concentrations were carefully applied along the internal sidewall of each glass jar inside a fume hood. Jars were immediately sealed and secured with a gas-tight metal canning lid and screw ring. Jars were placed in an incubator at 25 °C ± 1 °C and 70% R.H. with a 16 h photoperiod for 24 h. After 24 h, jars were opened and ventilated in the fume hood for 10 min. Vials were then removed from fumigation jars and placed into a desiccator chamber with a saturated NaCl solution at 22.5 °C ± 2.5 °C and 70% ± 2% R.H. for a recovery period of 72 h for mobiles and 168 h for eggs. Mortality of mobiles was determined by the inability to move, and eggs were scored as dead by failing to hatch to larvae. Any mobile stage that did not physically react following a 30-second viewing at the end of the recovery

period was considered dead. Fumigant concentration-response assays were replicated 2–8 times per concentration.

Time-Mortality Assays

Time-mortality assays were conducted with methods described above using the most tolerant life stage from the concentration-assays. Mites were tested at the concentration that consistently caused 100% mortality of the most tolerant life stage in 24 h, and the fumigant exposure time varied at time periods of 1, 2, 3, 4, 5, 6, 9, and 12 h. Time response assays were completed twice.

Verification of Fumigation Efficacy in Mixed Life Stage Colonies

The ability of the fumigants tested to kill individuals of *T. putrescentiae* in a concentrated population was evaluated in mixed life stage fumigations. Mixed life stage fumigations of mites used food bait plugs that were made and cut using methods similar to Amoah et al. (2016) for bait plugs used in mite traps. The only difference was an additional 10 g of agar were added to make the food bait plugs. The food bait plugs were placed in the center of a 118 mL glass jar. A Talkon® bristle paintbrush was used to gently brush the underside of the culture's filter paper lid into a tared 11 mL glass vial until 55 mg of mites (~7000 mites) were accumulated. The vial was deposited onto the food bait plug, and the jar was secured with 30 µm mesh (Fisher Scientific), a rubber band, and a metal ring. The rubber band secured the mesh above the metal ring to ensure water would not seep onto the mite mesh. Mixed life stage jars were placed into a soapy water bath and held for up to 12 h at 22.5 ± 2.5 °C and 70± 2% R.H.

The ability of the fumigants tested to kill individuals of *N. rufipes* in a small population was evaluated in mixed life stage fumigations using methods similar to Sağlam et al. (2015). Twenty-six adults from 6-week-old cultures were isolated using vacuum tweezers and a U.S. No.

20 sieve into a 118 mL glass jar that contained 7 g of Fishmeal sieved with a U.S. 40 sieve, 7 g of ground pet food sieved with a U.S. 40 sieve, and 7 g of whole pet food kibbles to create mixed life stage cultures. These cultures were then placed into an incubator set at 29 ± 1 °C with 70% R.H. 16:8 L:D. An additional 26 adults were added to each culture seven days later to create overlapping generations. All adults were removed with vacuum tweezers 35 d from the original infestation date, and colonies were left to develop in an incubator set at 29 ± 1 °C with 70% R.H. 16:8 L:D for a total of 75 d.

Mixed life stage jars of *T. putrescentiae* or *N. rufipes* were placed into a 1 L wide-mouthed glass jar containing an 11mL vial with 2 mL of water for humidity. Toxicant concentrations at 1 and 2x the concentration that consistently caused 100% mortality of *T. putrescentiae* in the initial fumigant concentration-mortality studies were applied to each fumigation jar with a 200–1000 µL micropipette. Iced liquid fumigant was applied along the inner side of each glass jar inside a fume hood as before. Jars were sealed and secured with a metal canning lid and canning ring. Jars were placed in an incubator set at 25 ± 1 °C with 70% R.H. and a 16 h photoperiod for 24 h. After 24 h, jars were vented in the fume hood for 10 min.

Mixed life stage colonies of *T. putrescentiae* were removed from fumigation jars and placed into a desiccator at 22.5 ± 2.5 °C and 70 ± 2% R.H. for a recovery period of 72 h to count the surviving mobile mites (protonymphs, deuteronymphs, tritonymphs, and adults). After mobile survival was counted, mixed life stage jars were placed into a 30 ºC water bath, and a 5 cm x 1 cm piece of paper was held to the food bait to remove the counted mobiles. The mixed life stage jars were placed back into the desiccator at previously described conditions until 168 h after the fumigation to count for egg hatch. *T. putrescentiae* mixed life stage fumigations were replicated five times.

Mixed life stage colonies of *N. rufipes* were removed from fumigation jars and placed into an incubator at 29 ± 1 °C with 70 ± 2% R.H. 16:8 L:D for a recovery period of 72 h to count surviving adults. After adult survival was determined, mixed life stage jars were placed into an incubator at 29 ± 1 °C with 70% R.H. 16:8 L:D, and adult emergence was counted every 14 d for 84 d. *N. rufipes* mixed life stage fumigations were replicated four times.

Sorption and Desorption

Sorption bioassays were conducted in labeled 2 L glass wide-mouthed jars that were loaded with 200 g of one of the following commodities: country ham, commercial dog food, or fishmeal, a mixed life stage jar, a 5 cm diameter glass Petri dish, and a glass vial containing 4 mL of water for humidity. The glass vial and the Petri dish were placed so that they were resting on the commodity. The mixed life stage jar of mites was set up as in the mixed life stage fumigations, with the exception that the jar was held for 48 h in a soapy water bath at room temperature in total darkness ~70% R.H to allow sufficient oviposition. PPO was added at 93.5 mg/L, and EF was added at 95.2 mg/L to the 3 cm diameter glass Petri dish. The 2 L jars were immediately sealed and secured with a metal gas-tight septum lid and placed in an incubator set at previous conditions for 12 h and 24 h; after which, the jars were ventilated for 10 min. Mixed life stage jars were then removed from fumigation jars and placed into a desiccator at 22.5 ± 2.5 °C, and 70% R.H. 16:8 L:D for a 72 h recovery period and checked subsequently in 168 h for egg hatch.

Desorption bioassays were conducted with the fumigated commodities of the sorption experiments; fresh untreated 1.8 mL vials containing a 3 mm^3 piece of ham and 20 mobile mites secured with 30 µm mite mesh (Fisher Scientific) and covered with a ventilated lid replaced the treated mixed life stage jars. The 2 L jars were sealed and secured with a gas-tight septum lid

and were placed in an incubator at 25 ± 1 °C and 70% R.H. Survivability was accessed as described in previous bioassays. Sorption and desorption bioassays were replicated four times.

Data Analysis

Fumigant concentration-mortality assays and time response assays were analyzed using probit regression SAS Studio® software (SAS 2018). Sorption and desorption mortality assay data were transformed with an angular transformation and tested for normality and individually analyzed using one-way ANOVA with general linear mixed models (GLMMIX) (SAS 2018). Means were separated using Tukey's HSD (Honestly Significant Difference) posthoc test at $P<0.05$.

Results

Fumigant Efficacy Results

Fumigant Concentration Response Assays

PPO and EF toxicity probit analysis results are reported in Table 3.1 and Table 3.2, respectively. The Pearson goodness-of-fit (χ^2) test showed that the probit regression model's fit to the observed data was significant ($P<0.05$) for all probit model regressions. Since the P-value for the tests was low, variances and covariances for each test were adjusted by a heterogeneity factor (Chi-square value (χ^2) divided by the degrees of freedom (df)), and the critical value from the t-distribution was then used to compute the fiducial limits for the LC_{50} and LC_{99} (SAS Institute, 2019). The mobile life stages were fully susceptible to both PPO and EF to the point that four concentrations could not be placed into a probit analysis to achieve a projected model. The average percent mortality is graphed against the achieved average concentrations for each target concentration in Figure 3.1.

Time Mortality Results

PPO could only achieve 56.6±6.6% mortality of *T. putrescentiae* eggs within a 12 h exposure at a fumigation rate of 71.42 mg/L (Table 3.3). However, EF achieved 100±0.0% egg mortality within a 6 h exposure at a fumigation rate of 70.09 mg/L (Table 3.3).

Fumigation Efficacy in Mixed Life Stage Colony Verification Results

Results of mixed life stage fumigations are reported in Table 3.4. *T. putrescentiae* averaged less than three mobiles to survive a PPO application at 93.5 mg/L and less than one mobile to survive at an application rate of 186.9 mg/L. *T. putrescentiae* averaged less than one egg to hatch to a larva at a PPO application of 93.5 mg/L and had no eggs hatch to larvae after an application at 186.9 mg/L. However, an application of EF at 95.2 mg/L resulted on average in no mobile survival and on average, with less than one egg hatch to larva. An application of EF at 190.4 mg/L on average resulted in no mobiles or eggs to survive. *N. rufipes* averaged less than two beetles to emerge after an application of PPO at 186.9 mg/L or EF at 190.4 mg/L for 84 d of observation. *N. rufipes* were controlled for 84 d with an application of PPO at 186.9 mg/L and with an application of EF at 190.4 mg/L.

Sorption and Desorption Bioassay Results

The results of sorption bioassays are depicted in Figure 3.2. The sorption of PPO did not cause significantly different survivability of mite larvae in the commodities for the 12 h exposure time (F=1.00, df=3, P=0.4262) or the 24 h exposure time. The sorption of PPO did not significantly impact mobile mite survivability with 24 h exposure time (F=1.10, df=3, P=0.3881), but survivability was significantly impacted with a 12 h exposure period (F=8.71, df=3, P=0.0024). The sorption of EF caused significantly different survivability of mite larvae with a 12 h and 24 h exposure time (F=52.39, df=3, P<0.0001), and (F=83.65, df=3, P<0.0001), respectively. Mobile mites that were exposed to EF for 12 h (F=3.84, df=3, P=0.0388) and 24 h

(F=4.13, df=3, P=0.0316) also had significantly different survivability. The desorption of either gas did not cause a significant difference in the survivability of the 1,254 PPO mobiles tested, nor the 1,285 EF mobiles tested during either exposure time.

Discussion

The tolerance of *T. putrescentiae* to the exposure of fumigants is traditionally eggs>mobiles (Kuverova and Stejskal 2009). Although an LC_{50} was not determined for mobile mortality in this study, the total mortality of the mobile stages was achieved within 24 h period using PPO at 15 mg/L and using EF at 10 mg/L. PPO and EF concentration-mortality data obtained in the current study did not fit the estimated probit models; this is likely due to variation in gas dispersal. Gas dispersal could have varied because the fumigations were held at normal atmospheric pressure and a temperature that was lower than the boiling point. This could have caused the gasses to condense and create concentrated toxic microhabitats (Phillips et al. 2012).

Due to this concern, fumigant concentration-mortality results were verified with heavily populated mixed life stage fumigations of *T. putrescentiae* and were verified by extending the monitoring of *N. rufipes* emergence for at least two generations over 84 d to confirm that selected concentrations for each fumigant could control each pest. Although sorption was not quantitatively measured for PPO or EF in fishmeal, ham, or pet food, results showed that different commodities have different sorption qualities, which can impact pest survivability. PPO and EF have been shown to have high sorption rates in several commodities (Zettler et al., 2003; Isikber et al., 2005; Darby et al., 2009). The accumulation of PPO was toxic enough to achieve mortality of *Plodia interpunctella* (Hübner) in peanuts and walnuts (Isikber et al. 2005), and now *T. putrescentiae*.

Results indicate that the liquid fumigant alternatives PPO and EF were effective at controlling *T. putrescentiae* and *N. rufipes* on selected commodities and that sorption of PPO and EF are independent of application rate. Future studies using commercially formulated PPO and EF should be tested to indicate if they are also able to control *T. putrescentiae* and *N. rufipes* with quantitative sorption and desorption assays on selected products, as well as testing for residual chemical in the products. These proposed studies would be an applicable approach to further support these liquid fumigants in receiving regulatory approval from government agencies as pesticides on more commodities in the U.S.

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Table 3.1 The Probit analysis of mortality response for *T. putrescentiae* eggs to tested concentrations of PPO during a 24 h exposure at 25°C.

Life stage	n	Slope ±SE	Intercept ±SE	LC$_{50}$ (F.L.) (mg/L)	LC$_{99}$ (F.L.) (mg/L)	χ^2	DF	$P > \chi^2$	R^2
Egg	416	1.28±0.63	0.08±0.57	0.87 (N.A.)	56.48 (N.A.)	54.48	18	<0.01	0.16

a The *P*-value smaller than 0.05 indicates a lack of statistically significant fit between the observed data and the expected regression line.

Table 3.2 The Probit analysis of mortality response for *T. putrescentiae* eggs to tested concentrations of EF during a 24 h exposure at 25°C.

Life stage	n	Slope ±SE	Intercept ±SE	LC$_{50}$ (F.L.) (mg/L)	LC$_{99}$ (F.L.) (mg/L)	χ^2	DF	$P > \chi^2$	R^2
Egg	1647	0.98±0.082	-0.74±0.11	19.74 (17.90 - 21.52)	96.17 (74.16 - 141.90)	210.69	31	< 0.01	0.19

a The *P*-value smaller than 0.05 indicates a lack of statistically significant fit between the observed data and the expected regression line.
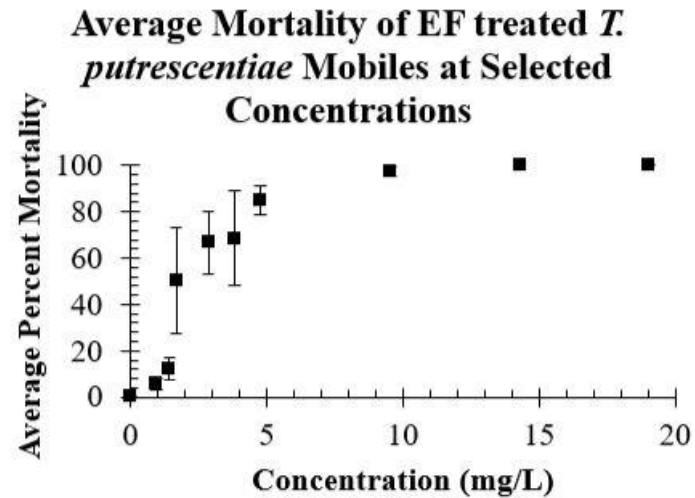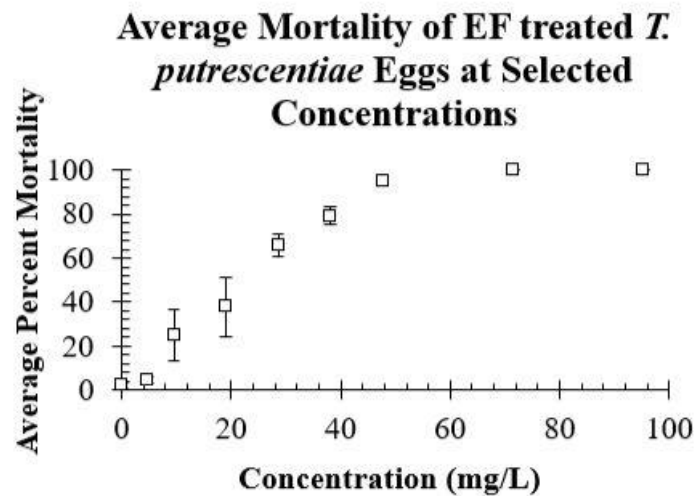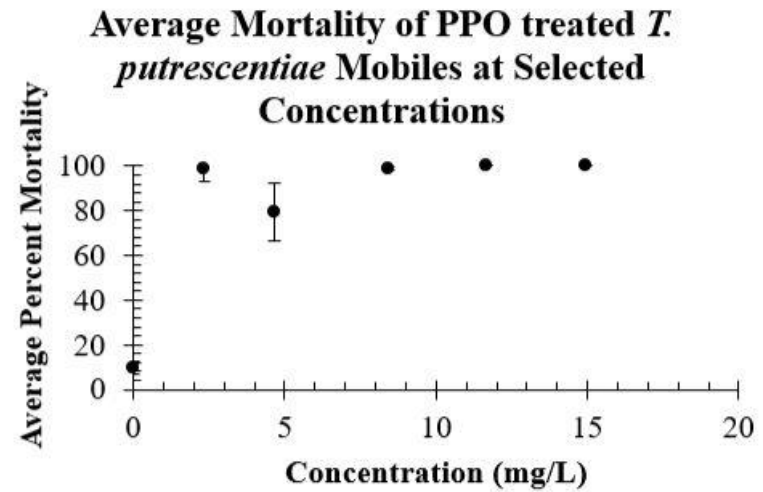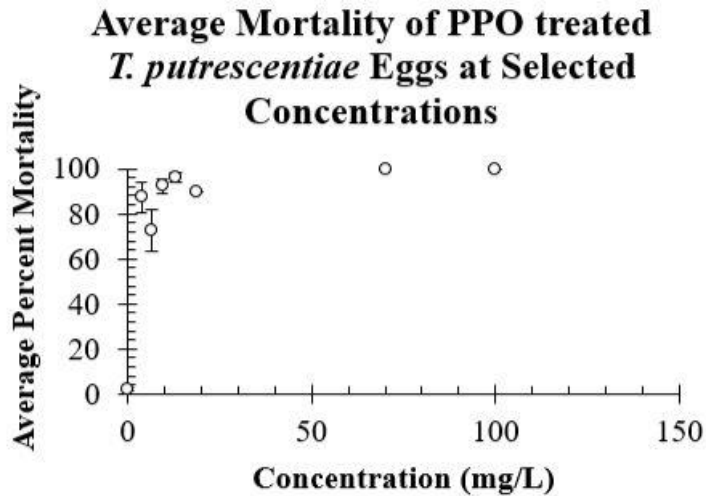
Figure 3.1 The selected PPO and EF concentration-dependent mortality of *T. putrescentiae* life stages exposed for 24 h at 25 °C. The top two graphs are of selected PPO concentration-dependent mortality (n= 2~6). The bottom two graphs are of selected EF concentration-dependent mortality (n=4).

Table 3.3 Average mortality response and standard error of *T. putrescentiae* at various exposure times to PPO and EF at 25 ℃.

Time (h)	Average Percent Mortality ± S.E.	
	PPO treated Eggs at 71.42 mg/L	EF treated Eggs at 70.09 mg/L
0	1.2±1.2	0.3±0.3
1	5.3±5.3	25.0±10.0
2	2.3±2.2	77.5±7.5
3	13.1±3.6	95.0±0.0
4	4.8±4.8	94.7±5.3
5	19.3±3.5	95.2±4.8
6	31.5±8.5	100.0±0.0
9	51.9±6.9	100.0±0.0
12	56.6±6.6	100.0±0.0

Table 3.4 The effects of two fumigants (PPO and EF) at two concentrations on the average population growths of *T. putrescentiae* (MM) and *N. rufipes* (RLHB) in mixed life stages in different recovery days post-fumigation.

Species Tested	Recovery Days	PPO			EF		
MM	3	0 mg/L	93.5 mg/L	186.9 mg/L	0 mg/L	95.2 mg/L	190.4 mg/L
	7	4431.5±1292.5	2.4±1.0	0.4±0.4	7884.5±587.5	0.0±0.0	0.0±0.0
		18704.5±442.5	0.6±0.4	0.0±0.0	15593.5±1491.5	0.6±0.4	0.0±0.0
RLHB	3	12.8±2.3	0.0±0.0	0.0±0.0	10.0±1.1	0.0±0.0	0.0±0.0
	14	4.0±0.7	1.5±0.6	0.0±0.0	4.0±0.7	1.5±0.5	0.0±0.0
	28	5.8±1.1	1.8±1.8	0.0±0.0	5.8±1.1	1.0±0.4	0.0±0.0
	42	2.8±1.3	1.3±1.3	0.0±0.0	2.8±1.3	0.0±0.0	0.0±0.0
	56	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	70	4.0±0.7	0.8±0.8	0.0±0.0	4.0±0.7	0.5±0.3	0.0±0.0
	84	3.8±0.9	0.0±0.0	0.0±0.0	3.8±0.9	0.0±0.0	0.0±0.0
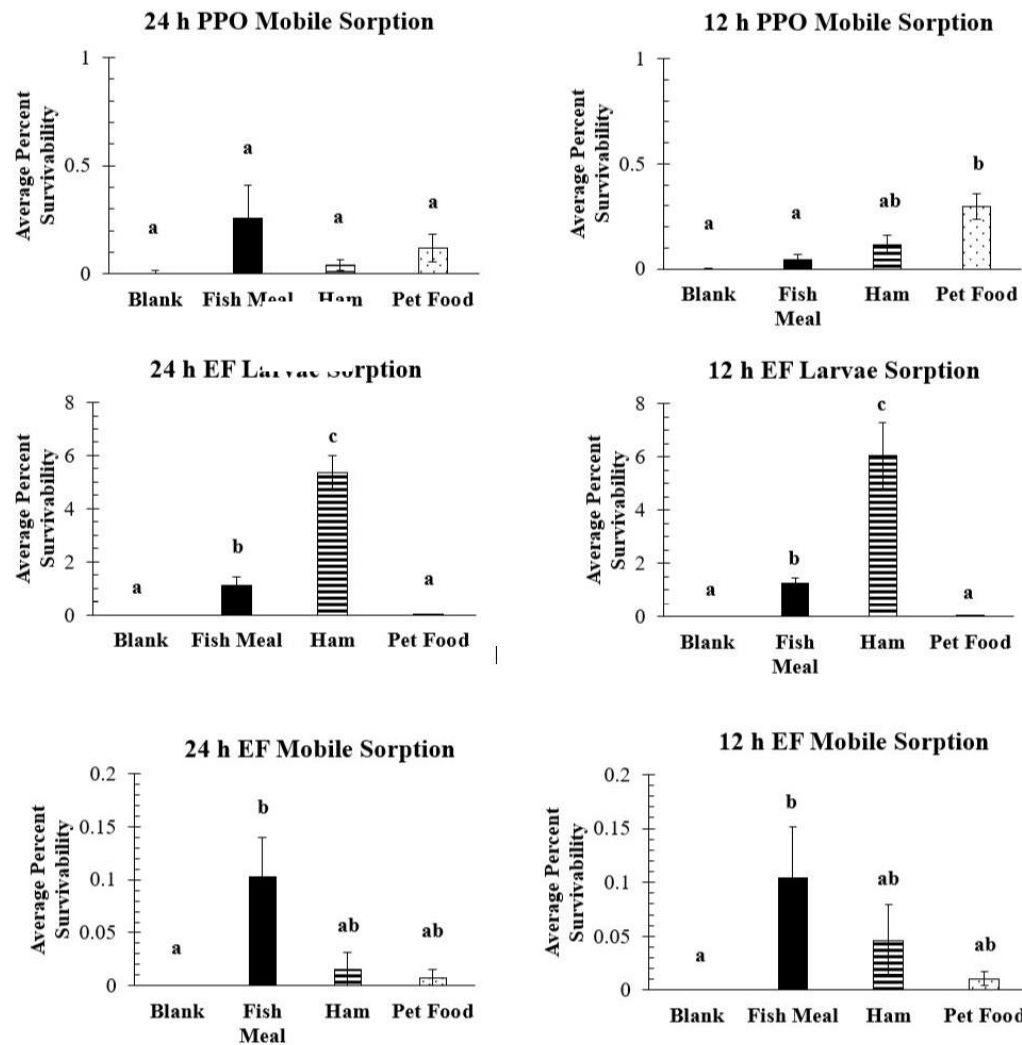
Figure 3.2 Sorption bioassays of average larvae and mobile percent survivability and standard errors on three commodities: country ham, commercial pet food, and fishmeal. The top two figures are PPO bioassays were conducted on *T. putrescentiae* mobiles (n=4) for 24 h and 12 h at 93.5 mg/L. The middle and bottom figures are EF bioassays conducted at 95.2 mg/L. The middle two figures are of *T. putrescentiae* larvae (n=4) survival, and the bottom two figures are of the mite mobiles (n=4) with exposure times of 24 h and 12 h.

Chapter 4 - Efficacy of Ethanedinitrile to Control *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae)

Introduction

Tyrophagus putrescentiae (Schrank, 1781) (Sarcoptiformes: Acaridae) is commonly known as the mold mite, the ham mite, or the copra mite and is associated as a pest of over 140 commodities (Hagstrum et al. 2013). Many of the commodities that *T. putrescentiae* prefers are composed of high-protein and high-fat contents such as dry-cured meats (Rentfrow et al. 2006), artisanal cheeses (Rentfrow et al. 2008, Krishnan et al. 2019), commercial pet food (Thind 2005, Brazis et al. 2008 Rybanska et al. 2016), and dried fruits (Dizlek et al. 2019). Infestations usually occur on the surface of a product; however, penetration of the commodity can occur (Zdárková 1991). Considerable economic damage can occur between infestation, penetration, and the mite's ability to cross-contaminate commodities with aflatoxin-producing fungi (Franzolin 1999).

Infestations are not readily detectable by sight and commonly persist unnoticed until a "dust" of mites' forms over the surface of the product, as they are of minute size with males ranging from 280–350 µm long and females ranging from 320–420 µm long. The progression of *T. putrescentiae* life stages is an egg, a six-legged larva, a protonymph, a tritonymph, and finally an adult (Vacante 2016). A generation of *T. putrescentiae* can be completed in 8–53 d, depending on the infested commodity, temperature, and relative humidity (Rinkikumari and Shukla 2019). Females begin laying eggs within 24 h of mating and can oviposit up to 500 eggs, which creates allows the pest population to grow exponentially (Boczek 1991). This mite causes extreme difficulties in developing facility management strategies especially in U.S. dry-cured hame products where there is a zero-tolerance of mite infestations (EPA 2011).

Management strategies that have been researched to reduce or eliminate *T. putrescentiae* have included sanitation, monitoring traps (Thind 2005, Amoah et al. 2016, 2017a), temperature

management (Abbar et al. 2016b), light type use (Amoah et al. 2017b), food-safe coatings (Abbar et al. 2015, Zhao et al. 2016, Campbell et al. 2017), food-grade netting (Zhang et al. 2017), residual pesticides (Abbar et al. 2016a), and alternative fumigants (Phillips et al. 2008, Zhao et al. 2015, Abbar et al. 2018). Fumigation is favored for *T. putrescentiae* control as it is ideal for penetrating products and areas that are otherwise inaccessible to control *T. putrescentiae* with minimal chemical alteration of the product or the addition of residues. Methyl bromide is a broad-spectrum fumigant that was used to control stored product pests for over 70 years until it was banned due to its ozone-depleting properties (Goupil 1932, Oberthür 1997).

In the U.S., methyl bromide is still used for quarantine and pre-shipment and critical use exemptions for California strawberry growers (EPA 2018). Phosphine is an alternative to methyl bromide, and it is effective at controlling *T. putrescentiae* (Zhao et al. 2015). However, phosphine's corrosive properties make it inappropriate for many storage facilities (Bond et al. 1984). Sulfuryl fluoride is another alternative to methyl bromide, but it is also an inadequate alternative (EPA 2018). Sulfuryl fluoride is ineffective in controlling *T. putrescentiae* eggs at room temperatures (Phillips et al. 2008). Therefore, alternative fumigants are necessary to investigate in order to find additional options for the rapid disinfestation of *T. putrescentiae*.

Ethanedinitrile (EDN) is an alternative fumigant that was patented as a broad-spectrum fumigant for use against insects, arachnids, nematodes, bacteria, molds, and rodents on a variety of commodities, including but not limited to grain, seeds, meats, fruit, vegetables, timber, and plants (O'Brien et al. 1999). The physical and chemical properties of EDN have been reviewed by Brotherton and Lynn (1959). EDN has a boiling point of -21 °C with a vapor pressure of 515 kPa at 21 °C (McConville and Swaminathan 2017). The national institute for occupational

safety and health (NIOSH) has a recommended exposure limit (REL) of 10 ppm (v/v), which compares favorably to both methyl bromide (5 ppm) and phosphine (0.3 ppm). Another benefit of this compound is that it is not an ozone-depleting substance (Ren et al., 2005). Although EDN can control stored product pests, *Rhyzopertha dominica* (F.) (Ren and Trang 2003, Hooper et al. 2003, Ren et al. 2014), *Sitophilus granaries* (L.) (Hooper et al. 2003), *Sitophilus oryzae* (L.) (Ren and Trang 2003, Hooper et al. 2003, Ren et al. 2014), *Tribolium castaneum* (Herbst) (Ren and Trang 2003, Hooper et al. 2003, Ren et al. 2014), *Tribolium confusum* du Val (Ren and Trang 2003, Hooper et al. 2003), *Ephestia cautella* (Walker) (Hooper et al. 2003), *Trogoderma variable* (Ballion) (Ren et al. 2014) and *L. serricorne* (F.) (Ren et al. 2014). However, studies to control *T. putrescentiae* are lacking. Therefore, the purpose of this study was to determine the efficacy of EDN fumigation at controlling *T. putrescentiae* by determining the concentration at which the most tolerant life stage would reach 100% mortality and verify the data through fumigations of large mixed life stage colonies in the laboratory.

Materials and Methods

Mite Cultures

Tyrophagus putrescentiae laboratory cultures have been maintained in the Department of Entomology at Kansas State University since 2008. Mite rearing methods followed were described as in Abbar et al. (2015). Mite cultures were reared in 1L glass wide-mouthed Ball© mason jars containing mite diet and then sealed with a labeled P8 Fisherbrand 9.0 cm diameter filter paper and the metal lid ring. The mite diet was composed of 75 g of a commercial dog food consisting of a minimum of 23% crude protein, 12% crude fat and 4% crude fiber at a 14% moisture content, and 475 mL water that were combined and heated for six minutes in a microwave oven (General Electric Co. 0.95 KW). The mixture was blended smooth in a 120 V

Hamilton Beach Blender©; then, the mixture was spooned into a one-liter beaker, where it was heated on a hotplate until boiling. Agar (ICN Biomedicals Inc.), alphacel (ICN Biomedicals Inc.), yeast (MP Biomedical LLC.), and Vanderzant modification vitamin mix for insect diets (MP Biomedicals LLC.) at a (5:5:5:5) g ratio and 25 mL of glycerol (Fisher) was added to the mixture and brought back to a boil. Then, 5 mL of 15% methyl-p-hydroxybenzoate in 95% ethanol (ICN Biomedicals LLC.) was added and heated for an additional 10 min. The cooked mixture was portioned evenly and combined with 14 g of the commercial dog food in mite rearing jars. Mites were introduced from a previous healthy culture after the diets were cooled to 25 °C and placed in a rearing cabinet at 25.5 ± 2.5 °C and 70-80% R.H. in darkness.

Mite Life Stage Separation

Healthy protonymphs, tritonymphs, or adults were classified as mobiles and selected using a one bristled natural bristle paintbrush from the culture's mass of mites located on the underside of the filter paper lid. The majority of mites selected were gravid adult females, and the gravid appearance distinguishes them with the eggs sometimes visualized within the female's idiosoma. The selected mites were transferred individually into a 1.8 mL glass vial containing a 3mm^3 piece of country ham. The vials were labeled and covered with a 30 μm mesh (Fisher Scientific), and a plastic ventilated lid. Vials were stored at 22.5 ± 2.5 °C and 70 ± 2% R.H. for up to 24 h before fumigation.

Mite mating for the isolation of eggs was initiated by selecting 50 healthy mobiles, mainly gravid females from the culture's filter paper lid. The mobiles were placed in a 118 mL glass jar with ~20 g of cooked mite diet mixture and covered with a labeled P5 Fisherbrand 7.0 cm diameter filter paper and a metal ring. The mobiles were left for 48 h in a double water bath of ambient temperature being ~25 °C and 70% R.H. in total darkness. Mite eggs were then

individually extracted using a one bristled Taklon® paintbrush and placed on double-sided sticky tape attached to a piece of black construction paper that contained a 3mm^3 piece of ham. The egg sheets were placed into labeled 1.8 mL vials, covered with 30 μm mite mesh (Fisher Scientific), and a plastic ventilated shell vial lid. Vials were stored at 22.5 ± 2.5 °C and 70 ± 2% R.H. for up to 3 h until fumigation.

Fumigant Concentration-Mortality Assays

Ethanedinitrile (Cyanogen, ≥ 99.90%) was supplied by Draslovka Services Pty Ltd., Sydney, Australia. Two-liter glass jars were loaded with 1.8 mL vials of 3mm^3 pieces of 6-month aged country ham (Brownsville, TN, USA) containing 20 mobiles or ten eggs per sheet, and 2 mL of water contained in an 11 mL glass vial. The fumigation jars were sealed with a modified gas introduction port gas-tight screw-on metal lid. The desired volume of gas was calculated based on the volume of the fumigation jar. Before fumigation, pure EDN was drawn from the cylinder into a 0.5 L Tedlar (polyvinyl fluoride) gas sampling bag (CEL Scientific Corp. California, USA). The desired volume of EDN to be added to each fumigation jar was then taken from the Tedlar bag using a gas-tight syringe (Hamilton, Reno, Nevada, USA) and then injected through the gas-tight septum after removing an equal volume of air from the jar. Fumigations were held for 24 h at 25 °C ± 1 °C with 70% R.H. and a 16 h photoperiod. Fumigation jars were aerated for one hour following fumigation. Vials were then removed from fumigation jars and placed into a desiccator containing a saturated NaCl solution at room temperature 22.5 °C ± 2.5 °C and 70% ± 2% R.H. for a recovery period of 72 h for mobiles and 168 h for eggs. Mortality of mobiles was determined by the inability to move, and eggs were scored as dead by failing to hatch. Any mobile stage that did not physically react following a 30-second viewing at the end of the recovery period was scored as dead. Fumigant concentration-

response assays were replicated in triplicate and analyzed using probit regression analysis SAS Studio® software (SAS 2018).

Fumigant Detection

Fumigant detection followed methods of Ramadan et al. (2019). Concentrations of EDN present in the fumigation jars were measured using a coupled gas chromatograph-mass spectrometer, GC-MS (Shimadzu17A QP5050A, Shimadzu Scientific Instruments, Kyoto, Japan) in the selected ion monitoring (SIM) mode with the electron impact (EI) ionization at 1.3 kV. The GC was equipped with a J&W Scientific DB-1MS UI (30.0 m x 0.250 mm I.D. with 0.25 µm phase thickness) capillary column (Agilent Technologies, Santa Clara, CA, USA). The carrier gas was ultra-pure helium at a flow rate of 1.8 mL/min. The oven temperature was isothermal and set at 150 °C. The injection port was set-up for split injection at 250 °C with the MS detector transfer line at the same temperature. The quantification of EDN was carried out in the SIM mode using 52 m/z. The concentration of EDN calculated for each treatment depended on an external standard curve generated just before each fumigation experiment. The standard curve was achieved by diluting a precise volume of the 100.0% EDN into a known volume of air in a Tedlar bag. GC-MS analyzed injections of this standard gas at volumes of 25, 20, 15, 10, and 5 µL. The area of the peak resulting from an injection of 15 µL was established to be equivalent to the standard concentration, while the other injection volumes represented proportionally smaller or larger amounts of EDN to generate the standard curve. The EDN concentration in each fumigation jar was injected three times from a headspace sample of 15 µL at the beginning and the end of the 24 h fumigation period. The three 15 µL EDN headspace samples for the beginning of the 24 h fumigation period was averaged and ending concentration

headspace samples were averaged. The beginning and ending average concentrations were averaged to give the concentration the fumigation jar held during the 24 h fumigation period.

Verification with Mixed Life Stage Fumigation

Food bait plugs were made and cut using methods described by Amoah et al. (2016). The only difference was that an additional 10 g of agar was added to make the food bait plugs. The food bait plugs were placed in the center of a 118 mL glass jar. A Taklon® paintbrush was used to gently brush the underside of the culture's filter paper lid into a weighed 11 mL glass vial until 55 mg of mites (~7000 mites) were accumulated. The vial was deposited onto the food bait plug, and the jar was secured with 30 µm mite mesh (Fisher Scientific), a rubber band, and a metal ring. The rubber band secured the mite mesh above the metal ring to ensure water would not seep onto the mite mesh. Mixed life stage jars were placed into a soapy water bath and held for up to 12 h at 22.5 ± 2.5 °C, and $70 \pm 2\%$ R.H. Mixed life stage jars were placed into a two-liter wide-mouthed glass fumigation jar containing an 11mL vial with 2 mL of water for humidity.

The fumigation jars were sealed with a modified gas introduction port gas-tight screw-on metal lid and fumigated at 300 ppm and 600 ppm with methods previously stated in the fumigant concentration-mortality assay methods. Concentrations of 300 ppm and 600 ppm were used because 300 ppm is where 100 % mortality was consistently seen for the most tolerant life stage, and 600 ppm is double that concentration within the fumigant-concentration mortality assays. Fumigated jars were placed in an incubator at 25 ± 1 °C with 70% R.H. and a 16 h photoperiod for 24 h. After 24 h, jars were vented in the fume hood for one hour. Mixed life stage colonies were removed from fumigation jars and placed into a desiccator at room temperature 22.5 °C $\pm$ 2.5 °C and 70% $\pm$ 2% R.H. for a recovery period of 72 h to count the surviving mobiles. After mobile survival was counted, mixed life stage jars were placed into a 30 ℃ water bath, and a 5

cm x 1 cm piece of paper was held just to the food bait to remove the counted mobiles. The

mixed life stage jars were placed back into the desiccator at previously described conditions until

168 h after the fumigation to count for egg hatch. Mixed life stage fumigations were replicated

five times.

Results

Fumigant Concentration Response Assays

Ethanedinitrile toxicity probit analysis results are reported in Table 4.1. The Pearson

goodness-of-fit (χ^2) test showed that the probit regression model's fit to the observed data was

significant (P <0.05) for all probit model regressions. Since the *P*-value for the tests were low,

variances and covariances for each test were adjusted by a heterogeneity factor (Chi-square value

(χ^2) divided by the degrees of freedom (df)), and the critical value from the *t*-distribution was

then used to compute the fiducial limits for the LC_{50} and LC_{99} (SAS Institute, 2019). The LC_{50}

in Table 4.1 suggests that eggs are the most tolerant life stage against ethanedinitrile. The

percent mortality was graphed against the average concentration for a range of selected target

concentrations (Figure 4.1).

Mixed Life Stage Fumigation Results

Control of *T. putrescentiae* was achieved at a fumigation rate of 600 ppm, and less than

0.05% of the population survived after a treatment at the fumigation rate of 300 ppm within a 24

h treatment at 25 °C (Table 4.2).

Discussion

Results show that *T. putrescentiae* exposure tolerance to ethanedinitrile (EDN) is higher

for the eggs than it is for the mobiles. This finding was expected because mite eggs are harder to

control than other mite life stages due to the egg's chorion being protected by a lipid-rich

secretion (Kucerova and Stejskal 2009) The EDN concentration-mortality data did not fit the

probit model; we attribute this to the 100 and 150 ppm range at which EDN is toxic to *T.*

putrescentiae mobiles and eggs, respectively. The narrow range causes difficulties with

accurately achieving target gas concentrations to be held for the duration of the exposure period.

However, the average emergence of *T. putrescentiae* from a 24 h EDN exposure at 600 ppm and

25 °C with a recovery period of 72 h for mobiles and 168 h for eggs was favorable for complete

control of both life stages. Mortality of the mites may be enhanced due to the high relative

humidity that is necessary to keep mites from desiccating, and the increased toxicity of EDN

applied under high humidities (Zhang et al. 2018, Hooper et al. 2003). Hooper et al. (2003)

reported enhanced mortality of *R. dominica* with an exposure of EDN at 0.33 mg/L for a 17-h

and 2 h exposure at various humidities. This study is the first known to measure EDN's efficacy

against *T. putrescentiae*.

There have been studies to demonstrate EDN efficacy on *R. dominica* (Ren and Trang

2003, Hooper et al. 2003, Ren et al. 2014), *S. granaries* (Hooper et al. 2003), *S. oryzae* (Ren and

Trang 2003, Hooper et al. 2003, Ren et al. 2014), *T. castaneum* (Ren and Trang 2003, Hooper et

al. 2003, Ren et al. 2014), *T. confusum* (Ren and Trang 2003, Hooper et al. 2003), *E. cautella*

(Hooper et al. 2003), *T. variable* (Ren et al. 2014) and *L. serricorne* (Ren et al. 2014). Studies

conducted by Ren and Trang (2003) were exposed to EDN for 20 or 40 min. at 25 °C. However,

studies conducted by Ren et al. (2014) were held exposed for 6 or 24 h at 25 °C and Hooper et al.

(2003) held exposure of EDN for 24 h at 30 °C. Unfortunately, details of the results conducted

by Ren et al. (2014) were not included; therefore, Hooper et al. (2003) studies will be used to

make comparisons of mortality responses. Although six species were investigated, three species

R. dominica, *T. castaneum*, *T. confusum* are most deeply studied. *R. dominica* adults and eggs

accomplished mortality after a 24 h exposure at 0.36 mgh/L or 166.54 ppm and 0.49 mgh/L or

226.67 ppm, respectively (Hooper et al. 2003). *T. castaneum* adults, eggs, larvae, pupae reached

100% mortality at the stated conditions at 1.1 mgh/L or 508.86 ppm, 1.3 mgh/L or 601.38 ppm,

1.2 mgh/L or 555.12 ppm, and 0.81 mgh/L or 374.71 ppm., respectively. *T. confusum* adults,

eggs, larvae, and pupae reached 100% mortality at the same conditions at 0.97 mgh/L or 448.72

ppm, 0.67 mgh/L or 309.94 ppm, 1.2 mgh/L or 555.12 ppm, and 1.3 mgh/L or 601.38 ppm,

respectively (Hooper et al. 2003). Results from this study indicate that *T. putrescentiae* reaches

100% mortality much like *T. confusum* at ~300–600 ppm.

Additionally, studies by Ren et al. (2014) reported that EDN was about 4.5 times more

toxic than methyl bromide for *T. castaneum* and twice as toxic to *T. variable* and *L. serricorne*,

this looks promising for control of *T. putrescentiae*. Future studies to determine fumigation

time-exposure, quantitative sorption, and desorption assays on selected stored products and the

addition of residual analyses could be beneficial before conducting commercial scale fumigation

studies. This research aides in the investigation of EDN as an alternative to methyl bromide by

controlling *T. putrescentiae*.

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Table 4.1 The Probit analyses of mortality response for *T. putrescentiae* life stages to tested concentrations of propylene oxide during a 24 h exposure at 25°C.

Life stage	n	Slope ±SE	Intercept ±SE	LC$_{50}$ (F.L.) (ppm)	LC$_{99}$ (F.L.) (ppm)	χ^2	DF	$P > \chi^2$	R^2
Egg	608	0.36±0.13	0.52±0.25	283.37 (164.71-8901)	4177 (789.07-396158022)	108.14	28	<0.01	0.20
Mobile	961	0.78±0.12	1.16±0.25	171.93 (N.A.)	484.20 (N.A.)	16488.96	22	<0.01	0.64

[a] The *P*-value smaller than 0.05 indicates a lack of statistically significant fit between the observed data and the expected regression line.
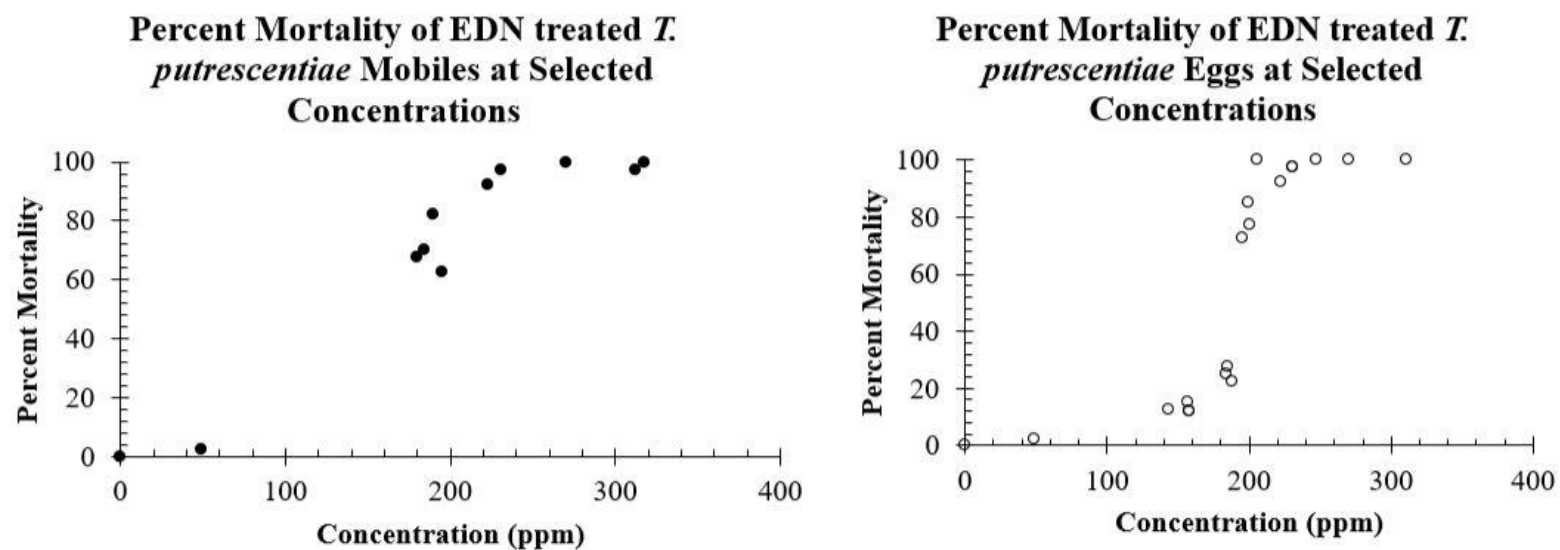
Figure 4.1 The dose response of *T. putrescentiae* life stages after selected EDN concentration 24 h treatments.

Table 4.2 The average and standard error of survived life stages in days post fumigation of ethanedinitrile (EDN) on *T. putrescentiae* mixed life stage fumigations at concentrations 1, and 2x the concentrations that cause 100% mortality (n=5).

Recovery Days	Emergence at Different Concentrations (ppm)		
	EDN		
	0 ppm	300 ppm	600 ppm
3	9758±1170	3.0±0.9	0.0±0.0
7	16360±771.5	5.0±2.1	0.0±0.0