Title

International Market Access for Commodities Impacted by Brown Marmorated Stink Bug, Halyomorpha halys (Stål), and Blueberry Maggot, Rhagoletis mendax (Curran)

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International Market Access for Commodities Impacted by Brown Marmorated Stink Bug, *Halyomorpha halys* (Stål), and Blueberry Maggot, *Rhagoletis mendax* (Curran)

By

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DISSERTATION

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Abstract

Quarantine insects can be major barriers to international trade if the commodities must be disinfested according to importer biosecurity regulations. Here we report case studies for the development of phytosanitary approaches to control blueberry maggot (BBM), *Rhagoletis mendax* (Curran), and brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), in regulated articles, horticultural and non-horticultural respectively. Blueberry maggot, a fruit fly native to Eastern North America, is a major pest of blueberries in this region that can prevent the trade of fresh blueberries across political borders, domestic and abroad. A novel method for quantitatively tracking the development of BBM in blueberries is described with application toward a systems-based approach for pest control at the probit 9 level, which includes a postharvest methyl bromide fumigation already familiar to industry. BMSB is an agricultural and urban pest that can cause damage to over 300 hosts. Overwintering BMSB, which enter diapause and aggregate in homes, vehicles, and other non-horticultural goods, are a major concern to Australia and New Zealand who demand imported consignments of refugia are fumigated. We report the efficacy of ethyl formate (EF), applied as a 16.7% by mass dilution in carbon dioxide, for controlling BMSB. A difference was identified in the relative tolerance between diapausing and non-diapausing BMSB, whereby non-diapausing BMSB are more tolerant to EF at durations less than 5.9 hours, but less tolerant than diapausing BMSB at longer durations.

Chapter 1: Conceptual themes

1.1. Quarantine insect pests and trade barriers

Globalization of trade, fueled by an extensive shipping network, has created unprecedented access to goods year-round, horticultural and non-horticultural products alike. Trade routes can transport insect "hitchhikers" in sufficient quantities to become established in ecosystems where they are not wanted. It is the purview of each country's National Plant Protection Organization (NPPO) to prevent the accidental importation of pests and maintain food security by creating and enforcing quarantines based on potential risks to economy and ecosystem. A quarantine is a regulatory restriction placed on the movement of goods or people to prevent the transportation of undesirable organisms until adequate control measures can be demonstrated and/or implemented. In the context of this dissertation, a quarantine pest is an insect of potential or realized economic importance that restricts trade.[1]

1.1.1. Fresh blueberries and blueberry maggot

Blueberries (*Vaccinium sp.*) were first domesticated in 1911 by Elizabeth Coleman White and USDA botanist Frederick Coville. Highbush blueberries quickly became popular in the US, and cross-breeding with lowbush blueberries expanded blueberry growing regions south to Florida and west to California, Washington, and Oregon. Currently in the US, blueberries are the second most produced berry, behind strawberries, and total blueberry production has been growing at a faster rate than strawberries.[2] From 2010 to 2019, the top 10 blueberry exporters increased their output from 148,096 tonnes to 473,875 tonnes,[3–5] with a total value of 2.3 billion $US in 2019.[3–7] Peru has also shown exponential growth, having just started blueberry production in 2013. Peru is now the largest exporter of blueberries with 125,056 tonnes of blueberries exported in 2019, more than double the US's 52,122 tonnes, and just 9,000 tonnes more than Chile's 116,000 tonnes.[4,5]

While Chile and Peru export far more fresh blueberries than the US, they are both located in the Southern Hemisphere, and thus have a reciprocal growing season relative to the US. Each hemisphere provides blueberries to the opposite hemisphere for year-round fresh blueberry availability. The Northern Hemisphere exported a combined 227,478 tonnes of blueberries in 2019 compared to 257,700 tonnes of blueberries exported in the Southern Hemisphere. [2–8]

Blueberries are highly perishable, and without cooling, blueberries become unfit for sale in less than one week. Forced-air cooling to near 0 °C and subsequent cold storage can preserve blueberry quality for 2-3 weeks without significant decay or loss of quality.[9,10] More recent advances in modified- or controlled-atmosphere techniques combined with proper packaging can extend the marketable shelf life of blueberries up to 8 weeks.[11–15]

With respect to the US, continual expansion of production area and development of high-yield blueberry varieties has saturated domestic fresh blueberry markets, and blueberry growers are pushing for greater international market access. The greatest expansion of production area has been in the Western US, where California, Washington, and Oregon increased production acreage by 8.5-, 5.0-, and 3.2-fold respectively from 2002 to 2017. Only blueberry production in Georgia has increased at a similar rate with a 4.1-fold increase in production acreage over the same time period.[16] Currently, California, Washington, and Oregon do not have blueberry maggot (BBM), *Rhagoletis mendax* (Curran), a fruit fly regulated as a quarantine pest, which allows the three states greater access to international markets. However, two key export markets, AUS and NZ, have withheld market access to Eastern and Western US blueberry growers until an acceptable phytosanitary treatment to control BBM in all regions is implemented.

Blueberry maggot is one of the top three pests of Eastern US orchards, alongside spotted wing drosophila (SWD, *Drosophila suzukii* (Matsumura)) and cranberry fruitworm (*Acrobasis vaccinii* (Riley)).[17] Spotted wing drosophila, like BBM, is currently regulated as quarantine pest for exports to AUS and NZ. A MB fumigation for SWD in fresh blueberries was developed by Walse and Tebbets showing probit 9 level control Currently, there is no approved phytosanitary treatment for BBM in fresh blueberries exported to AUS and NZ. One intent of this research was to determine the efficacy of the SWD treatment on BBM, because like Western US blueberry producers, Eastern US blueberry producers want access to AUS and NZ.

Toward this end, BBM biology must be understood, and a brief introduction follows. Blueberry maggot are typically univoltine and require a period of cold temperature to complete development.[18,19] Moreover, BBM females generally lay one egg per blueberry and use an oviposition marking pheromone to indicate to other females that the blueberry is already infested,[20] but multiple infestations can occur in rare, non-natural incidences of high insect pressure. BBM eggs progress through three instars before emerging from the blueberry to pupate in the soil between 5 – 15 cm depth. BBM then enter an overwintering diapause for ~ 90 days at temperatures consistent with fall and winter months in the Eastern US. Diapause is broken by increasing ambient temperatures, whereafter the pupa completes its maturation and emerges as an adult as blueberries nearby begin to ripen.

The majority of BBM emerge after 1 year of pupation, but ca. 10% will remain as pupae for 2 years and ca. 5% will remain pupae for 3 or more years.[18] The possibility of long-pupating BBM must be taken into IPM strategies as a latent BBM population can remain present for more than 3 years. Females typically emerge 5 days before males and begin to search for the protein sources required to fully mature. Male BBM require less protein than females. Adult BBM females will lay anywhere from 300 to 400 eggs. As they only lay one egg per blueberry, this is equivalent to 300 to 400 infested blueberries, and with zero-tolerance policies for many export markets, the presence of even a single gravid female can render an entire harvest unsalable.[19]

1.1.2. Brown marmorated stink bug refugia imported into Australia and New Zealand

Brown marmorated stink bug (BMSB, *Halyomorpha halys* (Stål)) is a pest that was recently introduced from Asia to North America. While BMSB is not yet a major horticultural pest in the Western US, it is a major pest in the Eastern US.[21] Adult BMSB will often overwinter outside of agricultural areas in non-horticultural consignments awaiting international shipment. In particular, vehicles and heavy machinery sales are dependent on oceanic shipping, and before being loaded into shipping containers, equipment can spend a significant amount of time in open lots where it can be infested by overwintering BMSB seeking refugia. As a part of their commitment to biosecurity, Australia (AUS) and New Zealand (NZ) regulate the importation of these non-horticultural commodities based on the presence of BMSB, particularly consignments shipped from the Northern Hemisphere in winter months, as overwintering BMSB do not require food during the 5 – 6 week voyage. In terms of US exports, AUS and NZ represented the 8th and 36th export markets for machinery and 14th and 30th for transportation equipment for a total of 10.9 billion $US in 2019, 2.6% of total US machinery and transportation equipment exports.[16]

1.2. Integrated pest management

Modern integrated pest management (IPM) is the spiritual successor to the 1959 integrated control concept (ICC) proposed by Stern, Smith, van den Bosch, and Hagen. In brief, the four authors described the process by which a particular insect species might rise to pest status, and the myriad of detrimental effects caused by the indiscriminate use of chemical controls. As an alternative they proposed the ICC, where the balance between insect pests and crops was an equilibrium, and that long-term control must be mediated by persistent biological factors, not just "temporary decimation of localized populations" as is the result of chemical controls.[22] In practice, however, sculpting a new dynamic ecosystem around a particular crop is impractical, but the concept of using multiple, targeted methods to control insect pests with minimal impact to the ecosystem became the basis for modern IPM strategies.[22] Chemical controls like pesticide sprays will likely remain a necessity in food production for the foreseeable future, however, the use of pesticide sprays should be informed by the chemistry and toxicology of the pesticide. An equilibrium exists between pesticide application and degradation. The chemistry of the degradation pathways for pesticides should be understood to limit the rate of application to avoid accumulation in the surrounding environment. The naturally occurring pesticide pyrethrum, extracted from chrysanthemum flowers, has been used as an indoor pesticide for over 2000 years, however it is rapidly degraded by sunlight and has limited usefulness in field applications. The structure of pyrethrins have been used as templates to develop analog molecules with the same mode of action called pyrethroids. Pyrethroids are designed to have increased stability to improve effectiveness, however those same modifications increase their environmental persistence.[23] As such, pesticide stability versus efficiency is a delicate balancing act.

Aside from pesticide sprays, chemical ecology can identify semiochemical signaling molecules and how they might be used to modify the behavior of organisms. For example, the structure of the male-produced aggregation pheromone for BMSB has been discovered and is already available as a commercial lures for traps.[24–26] Pheromones are highly selective and generally have low persistence thereby limiting non-target effects.

Research into IPM focuses on developing environmentally benign strategies that take full advantage of natural ecosystem factors such as biological controls, habitat manipulation, modification of cultural practices, and pest-resistant plant varieties. The ultimate goal of IPM is to develop a system of pest controls that works synergistically with the environment through a deep understanding of ecosystems and chemical ecology.

1.3. Postharvest methyl bromide fumigation and alternatives

Postharvest fumigation is used to disinfest commodities, horticultural and non-horticultural, which are defined as basic goods used in commerce. The United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) defines fumigation as "the act of releasing and dispersing a toxic chemical so it reaches the target organism in a gaseous state."[27] Any chemical that is not applied as a gas does not qualify as a fumigant under the USDA-APHIS definition. Aerosols, smokes, mists, and fogs are listed specifically as suspensions of particulate matter and not fumigants. USDA-APHIS also lists a set of ideal characteristics for a fumigant that should be easily and cheaply generated, easily detected by human senses, easily diffuses, rapidly penetrates commodity, harmless to foods and commodities, highly toxic to the target pest, inexpensive, insoluble in water, nonexplosive, nonflammable, nonpersistent, nontoxic to plants and vertebrates, and stable in the gaseous state. While no fumigant has all of these desirable properties, fumigants that are used or researched should be chosen based on maximizing the number of desirable properties.[27] The Food and Agriculture Organization of the United Nations' *Manual of Fumigation for Insect Control* by Monro offers similar, more practical guidelines for choosing a fumigant in that it should not be corrosive to fumigation chambers or shipping containers, should not leave irreversible residues, or otherwise injure growing plants, fruit or vegetables, or affect seed germination. Flammability and human toxicity are not necessarily grounds for disqualifying a fumigant given that risks can be sufficiently controlled.[28]

Commodities can be fumigated in storage, before departure, in transit, or upon arrival at their final destination. Dosage, temperature, duration, and atmosphere composition are the four major controls available to fumigators. Other conditions such as load factor, packing material, and headspace circulation are also important based on how they affect headspace concentration and distribution of the fumigant.

From 1945 to 1998 methyl bromide (MB) was the prominent fumigant for postharvest fumigations. Methyl bromide is a colorless gas that has a musty, acrid odor at high concentrations. Methyl bromide has a molecular mass of 94.94 g mol^{-1} with a vapor pressure of 1620 mmHg, and it is toxic toward insects and microbes (Table 1.1). However, MB catalyzes the destruction of ozone in the upper atmosphere, and in 1992, amendments to the Montreal Protocol instituted an international phase-out process for agricultural and structural uses of MB.[29] Many internationally traded commodities at the time were dependent on postharvest MB fumigations to meet quarantine disinfestation requirements, and fumigation research quickly focused on finding a replacement. Many alternative fumigants have been examined as replacements for MB since the Montreal Protocol such as sulfuryl fluoride, ethyl formate, phosphine, and hydrogen cyanide (Figure 1.1).[30–33] With an arsenal of potential fumigants, careful consideration of the chemical and physical properties is necessary when conducting fumigation research. Ethyl formate (EF), in particular, offers advantages over other fumigant choices with respect to non-target exposures. Ethyl formate has low mammalian toxicity compared to other fumigants and therefore poses less risk to workers and bystanders compared to other fumigants in Table 1.1.[34] These features of EF make it a promising candidate for relatively short fumigations of many different commodities, however the suitability of each fumigant should be considered situationally.

1.4. Systems-based approaches

The rapid growth of horticultural industry over the last 50 years has been largely dependent on synthetic pesticides to increase crop yields and postharvest MB fumigations to maintain international trade biosecurity. However, regulatory and consumer concerns over worker, consumer, and environmental exposures, which have been associated with deleterious effects, have become the centerpiece of (re)registration for synthetic pesticides and fumigants including postharvest MB.[35–42] As the list of available pest control strategies diminishes, concern over maintaining horticultural production and trade has grown. This difficulty is magnified for quarantine pests where pest-free status must be "guaranteed" according to the International Standards for Phytosanitary Measures 28.[43] The level of "guarantee" is dependent on the receiving country's NPPO with some countries requiring confirmation that no more than 32 individuals survive the treatment of 1 million insect while some other countries require that no survivors are observed after the treatment of 32,000 individuals. This benchmark is termed probit 9, which is equivalent to 99.9968% mortality.

A systems-based approach quantifies the combined efficacy of a series of strategies in order to meet quarantine regulations rather than relying on a single "standalone" treatment, such as a postharvest fumigation.[44–48] These quantitative, toxicological-based methods developed for systems-based approaches combines each protection event, from pre-plant to consumption, into a quantitative metric of insect control. By tuning each protection event, growers and regulators can continue to maintain, or even improve, biosecurity and commodity quality while reducing the dependence on pesticides or fumigants that might have harmful non-target effects. Systems-based approaches provide a basis for relieving industry and consumer concerns about the future of sustainable horticultural trade.

Table 1.1. Properties of some important fumigants.

Table 1.1

	Methyl bromide	Ethyl formate	Sulfuryl fluoride	Phosphine	Hydrogen cyanide
Molecular mass	94.94	74.08	102.06	34.00	27.03
Vapor pressure (25 °C; mm Hg)	1,620	200	13,000	30,150[a]	750
Water solubility (g L^{-1})	15.2 at 20 °C	136 at 20 °C	0.75 at 25 °C	0.31 at 17 °C	Soluble
Boiling point (°C)	3.56	54	-55.38	-87.7	26
Flash point (°C)	None	-20	None	Autoignition	-18
Toxicity (Rat; 4 h; ppm)	LC_{50} 405	LC_{83} 8,000	LC_{50} 500	LC_{50} 11	LC_{50} 158[b]
PEL TWA (8 h; ppm)	1	100	5	0.3	10

[a] Vapor pressure of phosphine measured at 21.1 °C

[b] mice; 1 h

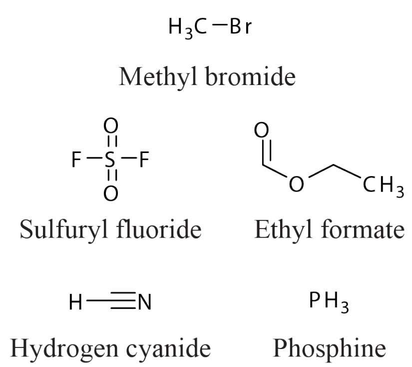


Figure 1.1 Structures of some important fumigants.

Part 1: Blueberry maggot, *Rhagoletis mendax* (Curran), case study

The primary objective of Part 1 was to quantify the efficacy of methyl bromide (MB) fumigation to control blueberry maggot (BBM, *Rhagoletis mendax*) using the existing method for spotted wing drosophila (SWD, *Drosophila suzukii*) in fresh blueberries. Chapters 2 and 3 lay the groundwork necessary to identify life stages of BBM that were fumigated inside fresh blueberries. Chapter 2 details the development and verification of the life history table. Chapter 3 examines the effect of rearing temperature on natural mortality used for the life history table and MB fumigations. Chapter 4 details the result of the MB fumigation and integration of that fumigation into a systems-based approach for controlling BBM.

Chapter 2: Life history table: A tool for retrospective life stage determination

2.1. Abstract

Blueberry maggot (BBM), *Rhagoletis mendax* (Curran), is a fruit fly native to Eastern North America that presents a serious barrier to fresh US blueberry exports and is regulated as a quarantine pest of blueberries by the Western US and most international trade partners. Blueberry maggot adult females lay eggs singly into blueberries, and a disinfestation treatment is required for the export of fresh blueberries from BBM infested regions. Due to its biology, BBM are difficult to rear in laboratory colonies, thus complicating the typical methods for developing disinfestation treatments. Additionally, BBM larvae exclusively feed internal to the blueberry complicating non-destructive identification of life stages. Pupae from Michigan were used to develop a life history table and subsequently a method for retrospectively determining the life

stage of internally feeding BBM present at any previous time (i.e., harvest, treatment) based on the observation of pupation. The retrospective life stage determination model was then validated using natural infestations of BBM in plantings from Southwest Michigan.

2.2. Introduction

Life history tables are developed for living organisms to quantify population dynamics such as developmental rate and mortality. In entomology, life history tables are often generated based on laboratory populations of insects that can be easily observed. The life spans of individuals in small cohorts are tabulated and then aggregated into one table. Individual variation is considered by directly observing each insect. However, the necessity of direct observation limits the applicability of life history tables to those populations which can be directly observed, and it excludes internally feeding insects.[49] Simplified life history tables can still be produced by ignoring immature instars and only measuring the time from oviposition to the emergence of the final immature life stage. However, where quarantine entomology is concerned, the effectiveness of a postharvest disinfestation treatment can affect individual life stages differently.[49] Therefore, identifying the relative distributions of blueberry maggot (BBM), *Rhagoletis mendax* (Curran), life stages present in blueberries at the time of fumigation was critical, as the most MB-fumigant-tolerant life stage relevant to blueberry marketing must be targeted in efficacy studies. There exist some methods for non-destructively observing internally-feeding larval instars such as X-rays[50] or detection of feeding-generated ultrasonic signals,[51] but they are expensive and require preliminary research to be applied to a specific insect. For an insect such as BBM, these existing methods lack the required sensitivity to detect larvae that are < 5 mm in length and have a density similar to their host. Destructive dissections can determine the developmental time span of individual life stages for internally-feeding insects at the expense of pooling the data and obscuring individual variability.[52]

A method for identifying the life stages for spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura), another fruit fly with internally feeding immature life stages, has been reported by Bellamy and Walse using a life history table at constant temperature.[53] That method, called the Developmental-Timespan Model, uses a controlled infestation period to determine when the fruit was infested, and the life stages present at fumigation are calculated based on the span of time between infestation and fumigation. The biology of BBM is such that laboratory rearing and controlled infestations are not feasible for fumigation development (Section 1.1.2.). Instead, pupation was used as an observable endpoint for development, and life stages present at fumigation were calculated retrospectively based on the span of time between fumigation and pupation. While dissections were used to enumerate the natural infestation levels (vide infra) and could potentially be used to gain some insight into the relative distribution of life stages present at the time of fumigation, the potential contribution of natural mortality, as defined in section 2.3.2.3, toward such distribution is better reflected when estimated retrospectively from temporal observation of pupation in non-treated controls of the same collection cohort, incubated at constant temperature (i.e., 26.6 °C and 80% RH). This approach accounts for natural mortalities, which are not necessarily equivalent across all BBM life stages, and more accurately estimates the number of BBM treated in fumigation trials.

A life history table was developed at the University of California, Davis Contained Research Facility (CRF) using BBM pupae collected from Michigan and incubated at a constant temperature of 26.6 °C. The weighted-mean occurrence and standard deviation of each life stage was calculated from the tabulated life history table data similar to the Developmental Timespan

Model.[53] The mean time to reach pupation from each life stage was used to identify each life stage retrospectively from the observation of pupation. The retrospective model was then validated using field-infested blueberries collected in Southwest Michigan by comparing the predicted emergence density curve and the density curve of observed pupae for multiple collection cohorts.

2.3. Materials and methods

2.3.1. Life history table

2.3.1.1. Insects and rearing

Blueberries infested with BBM were collected in August 2014 from naturally infested blueberry plantings at Southwest Michigan Research and Extension Center (SWMREC) in Benton Harbor, MI. After collection, pupae were placed in moistened vermiculite and transferred to an incubator set to 5°C for four months, environmental conditions favorable for the induction of overwintering. After the four months elapsed, pupae and vermiculate were shipped to the CRF at the University of California, Davis. Upon receipt, 400 pupae in moistened vermiculite were grouped and placed in a nylon and mesh enclosure (Bug Dorm-2®, BioQuip Products) housed in an incubator set to 26.6 ºC and 80% RH under a 16:8-h light:dark cycle (L:D). Adult emergence began approximately four weeks afterward, with a total of 181 flies emerging over the duration. Table sugar, vitamin mix (Vitamin Mix, diet fortification with choline, Bio-Serv®) and a 4:1 mixture of casein hydrolysate and minerals (Salt Mixture no. 2 USP XIII, MP Biomedicals LLP) were provided as diet.[54] Each of the diet components was presented separately on 90-mm plastic petri dishes lined with filter paper. Water was provided using a cup with an inserted cotton dental roll. Diet and water were replaced weekly.

2.3.1.2. Laboratory infestation

One week after emergence of adult BBM, ca. 30 blueberries were transferred into the enclosure. The blueberries were replaced daily to yield infestation cohorts spanning, and separated by, 24 h. After removal from the enclosure, blueberries were placed in larval cages made from plastic tubs (16 cup, Sterilite) with vented lids. Infested blueberries in larval cages were suspended on a plastic grid over a layer of moistened sand (Premium Play Sand, Quickrete®), which provided a pupation substrate for larvae emerging from fruit. Larval cages were held in the rearing incubator alongside adult enclosures. Every 48 hours, a set of three blueberries was randomly sampled from each cage and dissected to determine the number and life stage of the specimens present to track the chronology of development, or stage frequency data, in a life history table. Additionally, after each dissection, sand from each cage was sifted to locate any pupae and/or newly emerged third instar larvae. All collected pupae and larvae were placed in petri dishes with moistened vermiculite and held in the rearing incubator until all pupated. Dissections and sifting were terminated when no more specimens were encountered. A total of 759 blueberries were presented for infestation and a total of 560 larvae and pupae were collected. Of the 759 blueberries presented, 74% were infested and 8% contained multiple BBM pupae.

2.3.1.3. Calculations

Stage-frequency data,[55] such as that common to life history tables, allows for the calculation of the probability that a particular BBM life stage was present after oviposition, such as at the time of treatment. There are a couple assumptions for this method: 1) The development time between life stages follows a normal distribution, and 2) there is no mortality across development in the life history table dataset. By assuming that the development time of each life stage follows a normal distribution, the time to develop from one life stage to another life stage can be calculated as the difference between the means with appropriately propagated standard error.

First the weighted mean (eq. 2.1) in days, for an egg to reach each life stage ($\bar{x}_{first}$, $\bar{x}_{second}$, $\bar{x}_{third}$, $\bar{x}_{pupa}$) was calculated from the life history table:

$$\bar{x}_{LS} = \frac{\sum_1^t \left(t * n_{LS}(t)\right)}{\sum_1^t n_{LS}\,(t)}$$

(eq. 2.1)

where *t* is the day where a life stage was observed and $n_{LS}(t)$ is the number of that life stage (LS) observed on day *t*. Then the weighted variance (s_{LS}^2) (eq. 2.2) was calculated with correction for weighting where df_{eff} is the corrected number of degrees of freedom:

$$s_{LS}^2 = \frac{\sum_{t=1}^t n_{LS}(t) * (t - \bar{x}_{LS})^2}{\sum_{t=1}^t n_{LS}(t)} * \frac{df_{eff}}{df_{eff} - 1}; \; df_{eff} = \frac{\left(\sum_{t=1}^t n_{LS}(t)\right)^2}{\sum_{t=1}^t (n_{LS}^2(t))}$$

(eq. 2.2)

The mean time for each life stage to be observed as a pupa ($\overline{D}_{LS}$, eq. 2.3) was calculated:

$$\overline{D}_{LS}(\pm 1.96\sigma_{LS}) = \bar{x}_{pupa}(\pm 1.96 s_{LS}) - \bar{x}_{LS}(\pm 1.96 s_{LS})$$

(eq. 2.3)

Standard deviations were propagated according to Skoog and Leary[56] for non-independent data. The mean time for an egg to be observed as a pupa is a special case where $\overline{D}_{egg} = \bar{x}_{pupa}$. The weighted mean occurrence of each life stage is tabulated in Table 2.1.

2.3.1.4. Retrospective analysis

The occurrence of a life stage can also be estimated retrospectively, such as at the time of treatment, from the observation of pupation, or adult emergence based on an understanding of stage-frequency data as described in Bellamy and Walse.[53,57] The probability that an observed pupa was a particular life stage at time of treatment, P_{LS}, was calculated from the normal distribution (eq. 2.4) where $\mu = \overline{D}_{LS}$, $\sigma = \sigma_{LS}$, and $x = t_r$, the time (*n* days) between the observation and retrospective treatment.

$$P_{LS}(t_r, \overline{D}_{LS}, \sigma_{LS}) = \frac{1}{\sigma_{LS}\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{t_r - \overline{D}_{LS}}{\sigma_{LS}}\right)^2}$$

(eq. 2.4)

The probabilities for eggs and third instars are special cases where $P_{egg}(t_r) =$ cumulative distribution function (cdf) and $P_{third}(t_r) = 1 -$ cdf. From here, the probability of a life stage occurring each day (t_r) before the observation of pupation ($P'_{LS}(t_r)$, eq. 2.5) was normalized such that the sum probability of all life stages is 100%:

$$P'_{LS}(t_r) = \frac{P_{LS}(t_r)}{\sum_{LS=egg}^{third} P_{LS}(t_r)}$$

(eq. 2.5)

If a single pupa was observed, it was assigned to the life stage with the highest probability. If multiple pupae were observed on the same day, they were assigned to life stages by multiplying the number of pupae found by the probability of each life stage and rounded to the nearest integer. For example, if a pupa was observed 13 days after fumigation $t_r = 13$, $P_{egg}(13) = 0.029$, $P_{first}(13) = 0.095$, $P_{second}(13) = 0.033$, and $P_{third}(13) = 0$.

$$P'_{first}(13) = \frac{0.095}{0.157} = 0.605$$

Any pupa found 13 days after fumigation had a 19% chance of being an egg at the time of treatment, a 60% chance of being a first instar, and a 21% chance of being a second instar. If 8 pupae were observed 13 days after fumigation, 2 would be assigned as eggs, 4 would be assigned as first instars, and 2 would be assigned as second instars. As such, this model was applied to predict the probability distribution of life stages at the time of treatment based on pupation from non-treated, naturally infested blueberries (vide infra).

Mathematical terms	
LS	Life stage
$\bar{x}_{LS}$	Weighted mean number of days to reach each life stage from oviposition
s_{LS}	Weighted standard deviation of $\bar{x}_{LS}$
t	Day of index "*i*" where a life stage was observed
$n_{LS}(t)$	Number of life stages observed on day, *t*.
df_{eff}	Effective degrees of freedom for calculating s_{LS}
t_r	Number of days retrospective to the observation of a pupa
$\bar{D}_{LS}$	Mean time for a life stage to reach pupation
σ_{LS}	Propagated standard deviation of $\bar{D}_{LS}$
$P_{LS}(t_r)$	The probability of a life stage occurring on day, t_r
$P'_{LS}(t_r)$	The normalized probability of a life stage occurring on day, t_r
r_i	Infestation rate
r_E	Rearing efficiency

2.3.2. Application of retrospective model: field verification

2.3.2.1. Fruit collection

Blueberries were collected over multiple years from a planting near Bangor, MI last operated commercially in the early 2000's, and SWMREC in Benton Harbor, MI. All blueberries collected on the same day from the same location were labeled by date, as a cohort. Blueberries were either handpicked into buckets from throughout the field or from designated rows, and then in both cases evenly distributed by hand from each of the buckets to fill 6 oz. clamshells (Mid-Lantic Labeling and Packaging) (Table 2.2).

Specific to each collection date, three clamshells were randomly selected and reserved for dissection to reference the chronology of development in the collection cohort relative to the stage-frequency metrics of the previously established life history table (vide supra). Clamshells were then emptied and the blueberry contents of two clamshells were transferred into each rearing container. All rearing containers were then placed into an incubator at 26.6 °C and 80% RH.

2.3.2.2. Rearing containers

Rearing containers consisted of two modified plastic cups nested together. The bottom cup was a 48 oz. square deli container (US Plastic) with 2 opposing vent holes (2-cm dia.) cut in the sides and covered with a breathable carbon-fiberglass mesh (Charcoal Super Solar Screen, Phifer Inc.). Damp play sand, 2 – 5 cm deep, (Premium Play Sand, Quickrete®) was added to the bottom cups. The top cup was a 32 oz. square deli container (US Plastic) that had the bottom replaced with ¼-in. hardware cloth to retain the blueberries but allow larvae to pass through to the damp sand below. The lids had a single 5-cm dia. vent hole covered with the same carbon-fiberglass mesh as the vent holes in the bottom cup.

2.3.2.3. Natural infestation and mortality

Blueberry maggot infestation levels were determined through dissections as aided through microscopic inspection of ca. 300 blueberries for each collection cohort that were taken from the three clamshells previously set aside. The number of each egg and larval BBM instar was recorded (Table 2.3). The "infestation rate" (r_i) was defined as the sum of BBM eggs and larval instars recorded divided by the number of blueberries dissected (i.e., 300). The "rearing efficiency" (r_E)

was defined as the number of pupae observed divided by the number of blueberries held at rearing conditions. r_i and r_E were calculated for each cohort (Table 2.2). Percent natural mortality was calculated as $(1 - r_E/r_i) * 100\%$.

2.3.2.4. Pupae Collection

Every 24-48 h, groups of rearing containers were removed from incubators. The top and bottom cup of each container was separated, and the top cup was set aside while the sand in the bottom cup was poured into a hemispherical kitchen strainer (ca. 18 mesh or ca. 1 mm holes). The kitchen strainer was then partially lowered into a bucket of water until the sand was washed the sand through the strainer. Pupae and third instar larvae were counted and recorded for each container before being transferred into a separate plastic cup containing moist play sand. New play sand was then added to the bottom cup and re-nested with the top cup before being returned to the incubator. Calculations of r_E were conducted for each cohort as the number of pupae observed divided by the number of blueberries.

2.3.2.5. Statistical testing

Twenty blueberry cohorts were used to confirm that the life history calculations could accurately predict the temporal profile of pupal emergence from the naturally infested blueberries. The results from the dissections immediately after collection for each cohort were projected and compared, using a Welch's *t* test ($t_{critical} = 1.96$), to the observed temporal distribution of pupation associated with each cohort.

The "Total" column in Table 2.4 is the result of the Welch's *t* test between the predicted emergence density curve and the density curve of observed pupae for that collection cohort. The total column was the only set of comparisons that depended on the proportion of life stages observed in dissections, wherein a higher proportion of earlier (egg or first) or later (second or third) instars would affect the mean of the total predicted temporal distribution of pupation (Figure 2.1). The other columns in Table 2.4 are the results of the Welch's *t* test for each life stage. Density curves that are statistically dissimilar, or non-passing, are highlighted in bold, and "NA" entries indicate no egg or larva of the specified life stage was observed during dissections, so no comparison could be made.

2.4. Results and discussion

2.4.1. Natural mortality in field-infested blueberries

The r_i ranged from 22 to 57% BBM, while r_E ranged from 4 to 64%. The levels of mortality in naturally-infested berries ranged from 11% to 90%. However, in 5 of the 20 cohorts, r_i was less than r_E, and more BBM pupae were reared than predicted by r_i (Table 2.2). Dissections of more blueberries could have improved estimates of the "true" BBM infestation rate, however, at a rate of 2 – 3 minutes per blueberry per person the time required to dissect more than 300 blueberries was exhaustive. r_E can be used instead to approximate natural mortality, assuming that r_i remains approximately constant.

Blueberry maggot r_E in Bangor, MI appeared to follow a decreasing trend throughout the growing seasons of 2018, 2019, and 2020 (Table 2.2). The linear least-squares regressions of r_E versus time in 2018, 2019, and 2020 had slopes of -4.3, -1.1, and -1.5% d^{-1}. Regressions of r_E in 2018 and 2019 were surprisingly linear with R^2 values of 0.889 and 0.969 respectively, while the regression in 2020 was much less clear with an R^2 of 0.188. Spotted wing drosophila co-infestation and fruit quality both appeared to have a correlation to immature BBM mortality. In 2018, very few SWD larvae were initially observed in Bangor, MI cohorts, but SWD numbers increased throughout the growing season, while in 2019 and 2020, SWD co-infestation was abundant early and continued to increase. While the decline in fruit quality appeared to be consistent from year to year, SWD pressure in 2018 was less than in 2019 and 2020. This difference could explain the steeper r_E versus time regression slope for 2018 compared to 2019 and 2020. Fewer cohorts were collected from SWMREC, however in 2019, SWD co-infestation was prevented by enclosing blueberry rows in nets, and blueberry maggot pupae from the previous season were "seeded" into the netted rows. Without SWD co-infestation, r_E in the SWMREC North Net remained similar between August 6 and August 15 at 17% BBM and 19% BBM respectively (Table 2.2). Blueberry maggot females lay a single egg per blueberry and do not infest blueberries already containing an egg. Immature BBM then require ca. 17.6 days from infestation to emerge from the blueberry as a pupa and consume most of the blueberry flesh to complete development. Female SWD, by comparison, will infest blueberries regardless of prior infestation, and immature SWD can complete development to pupation in ca. 7 days.

2.4.2. Retrospective analysis of natural infestation

Of the 83 comparisons (NAs excluded), 77 passed the Welch's *t* test using a *t* critical value of 1.96 and did not reject the null hypothesis that the means are the same (Table 2.4). Of the six tests where the null hypothesis was rejected, four of those comparisons were for the total pupal emergence, and the other two comparisons were life-stage specific for eggs and first instars (Table 2.4). Inherently, variation in each successive life stage is additive (Table 2.1). Thus, the total pupal emergence will have the widest distribution, as variation for each life stage is compounded. Under the same conditions, natural variability observed in rearing experiments should be reflected in the life history table, however SWD co-infestation reduces the suitability of the blueberry for BBM development which can increase the dispersion of life stages.[58–60] Additionally, SWD co-infestation, which leads to near 100% mortality of younger instars, can drastically shift the weighted-mean of total pupal emergence used in the Welch's *t* test (Figure 2.1).

2.4.3. Method efficiency

Using field-infested blueberries greatly increases research productivity and efficiency for BBM relative to lab conditions, even when considering SWD and the other complicating factors discussed. From the 400 BBM pupae provided by MSU, only 181 BBM adult flies emerged. That cohort of adults was presented 759 blueberries to produce 560 BBM pupae and larvae. By contrast, blueberry cohorts from Michigan had a mean r_E of 25%, including cohorts impacted by high SWD co-infestation. Using the mean r_E of 25% as a conservative estimate, the effort required to collect 100,000 blueberries was trivial compared to laboratory rearing methods and would produce 25,000 BBM pupae. r_E could be, and was, further improved by installing exclusion netting to prevent SWD co-infestation, discussed further in Chapter 3.

2.4.5. Other retrospective models

Life history tables are frequently used in entomology to describe and predict population dynamics of insect, and in forensic entomology, they can be used to determine the postmortem interval. The Developmental Timespan Model (vide supra) also uses host-specific life history tables to predict distributions of life stages in both a forward and retrospective manner, however the Developmental Timespan Model and postmortem interval both depend on a simultaneous starting point for retrospective determinations.[53,61–64] The retrospective model presented here decouples the rates of development from a simultaneous starting point and requires only a developmental endpoint. Decoupling from an initial starting point increases estimation variance through propagated error and can limit the accuracy of life stage determinations, therefore accurate life stage determinations depend on limiting developmental variability. Both host suitability and temperature variation contribute to developmental variability.[58–60] Temperature variations can be easily controlled, but for oligophagous insects identifying an optimal host can prove challenging.[57] This makes *Rhagoletis sp.* exceptionally well suited to this method as they are monophagous and develop internally to their host, preventing the use of direct observation methods. Of the 72 species in the *Rhagoletis* genus,[65] BBM, Eastern cherry fruit fly (*R. cingulata*, Loew), black cherry fruit fly (*R. fausta*, Osten Sacken), and walnut husk fly (*R. completa*) are of the most economic importance.[19,66–68]

2.5. Conclusion

The retrospective analysis of field-infested blueberries to determine the life stages present at the time of collection based on the observation of pupae is a critical tool for the study of *Rhagoletis sp.*, such as BBM and other difficult-to-rear, univoltine, internally feeding insects. The method detailed here shows that an isothermal life history table can be used in conjunction with predictive statistics to increase the numbers of insects that can be studied without needing to maintain colonies.

Table 2.1. Weighted mean occurrence of each immature blueberry maggot life stage.

The weighted mean occurrence in days ($\bar{x}_{LS}$), weighted mean time to pupation ($\bar{D}_{LS}$), and associated standard deviations (s_{LS}) were calculated for each immature blueberry maggot life stage from tabulated life history table data. The time for an egg to reach pupation was 17.6 days, $\bar{D}_{egg} = \bar{x}_{pupa} = 17.6\ days$.

Table 2.1

Life stage	$\bar{x}_{LS}$	s_{LS}	$\bar{D}_{LS}$	s_{LS}
Egg	4.3	1.7	17.6	2.5
First	7.0	1.8	10.6	3.1
Second	9.7	2.0	7.8	3.1
Third	13.5	2.2	4.0	3.3
Pupa	17.6	2.5	NA	NA

Table 2.2. Blueberry collection and pupae rearing numbers.

Blueberries were collected from the Southwest Michigan Research and Extension Center (SWMREC) and an abandoned blueberry orchard in Bangor, MI. For each cohort, defined as blueberries collected on the same date, the number of blueberries (BB) collected, pupae reared, and pupae expected are tabulated. Rearing efficiency, r_E, and infestation rate, r_i, were calculated as the percentage of blueberries reared or dissected that produced a pupa or contained a larva, respectively. Percent natural mortality was calculated as $(1 - r_E/r_i) * 100\%$. Negative natural mortality indicates more pupae reared than expected.

Table 2.2

Cohort	Location	Picked	BB reared	Pupae reared	Pupae expected	r_E	r_i	Natural mortality
C1-2018	Bangor	12-Jul	2208	1416	824	64%	37%	-72%
C2-2018	Bangor	15-Jul	1891	1032	736	55%	39%	-40%
C3-2018	Bangor	18-Jul	1596	819	788	51%	49%	-4%
C4-2018	Bangor	22-Jul	1327	252	566	19%	43%	55%
C5-2018	SWMREC	31-Jul	1482	342	432	23%	29%	21%
C1-2019	Bangor	23-Jul	901	89	351	10%	39%	75%
C2-2019	Bangor	25-Jul	285	23	99	8%	35%	77%
C3-2019	Bangor	27-Jul	678	31	252	5%	37%	88%
C4-2019	Bangor	29-Jul	463	19	174	4%	38%	89%
C5-2019	SWMREC	6-Aug	3459	605	755	17%	22%	20%
C6-2019	SWMREC	15-Aug	1591	260	583	16%	37%	55%
C7-2019	SWMREC	19-Aug	1559	549	488	35%	31%	-12%
C1-2020	Bangor	14-Jul	1792	277	747	15%	42%	63%
C2-2020	Bangor	15-Jul	1920	91	910	5%	47%	90%
C3-2020	Bangor	17-Jul	2169	191	1043	9%	48%	82%
C4-2020	SWMREC	19-Jul	1838	356	925	19%	50%	62%
CN-2021	SWMREC	12-Jul	1125	593	645	53%	57%	8%
CS-2021	SWMREC	12-Jul	1125	577	311	51%	28%	-86%

Table 2.3. Dissection results and predicted life stages.

Results of initial dissections of blueberries (BB diss) were recorded, and the number of each life stage observed was tabulated. Rearing results indicate the number of each life stage that was predicted based on the dissection results and the retrospective model.

Table 2.3

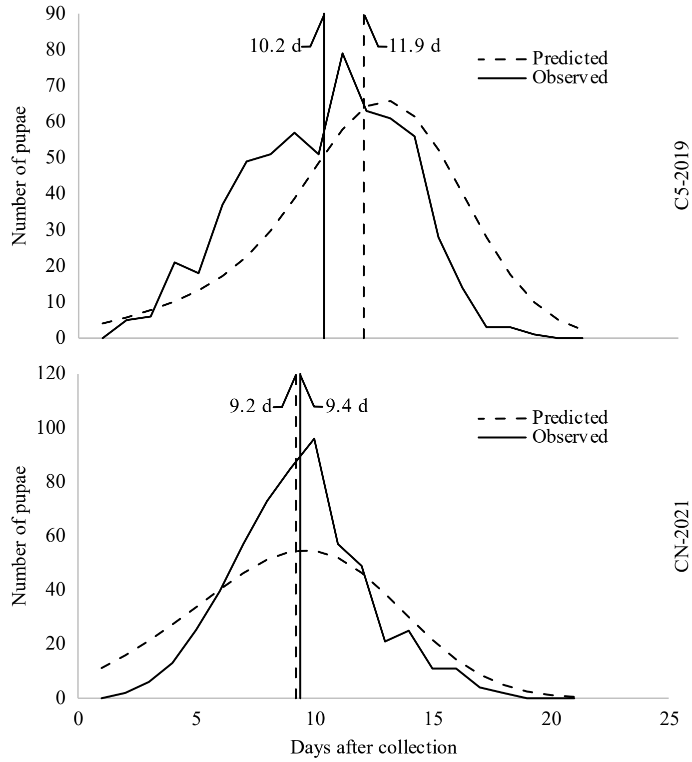
		Dissection results					Rearing results			
Cohort	Picked	BB diss	Egg	1st	2nd	3rd	Egg	1st	2nd	3rd
C1-2018	12-Jul	300	51	32	29	0	212	717	407	80
C2-2018	15-Jul	298	23	37	47	9	50	420	395	167
C3-2018	18-Jul	322	19	37	71	32	150	237	267	165
C4-2018	22-Jul	300	24	26	32	46	9	33	78	132
C5-2018	31-Jul	350	9	22	37	34	4	72	119	147
C1-2019	23-Jul	267	21	37	35	10	0	20	34	35
C2-2019	25-Jul	300	8	37	39	20	0	5	8	10
C3-2019	27-Jul	301	2	31	55	24	0	8	12	11
C4-2019	29-Jul	301	6	51	41	15	0	4	7	8
C5-2019	6-Aug	403	61	14	8	5	78	252	190	85
C6-2019	15-Aug	292	10	37	41	19	14	80	93	73
C7-2019	19-Aug	300	5	21	54	14	44	200	196	109
C1-2020	14-Jul	300	58	49	18	0	15	127	103	32
C2-2020	15-Jul	287	76	35	25	0	1	32	37	21
C3-2020	17-Jul	264	50	54	22	1	7	78	74	32
C4-2020	19-Jul	292	43	46	38	20	5	89	136	126
CN-2021	12-Jul	75	4	20	12	7	19	224	228	121
CS-2021	12-Jul	76	3	9	6	3	42	229	209	97

Table 2.4. Welch's *t* test results.

A Welch's *t* test was used to test the null hypothesis that there is no difference between predicted and observed distributions of observed pupae for each cohort. Results of the Welch's *t* test are reported as the *t* critical value. Values over 1.96 (95% confidence) where the null hypothesis was rejected are bolded. Values with "NA" did not have any predicted distribution due to a lack of observed life stages in dissections.

Table 2.4

		Welch's *t* test results				
Cohort	Picked	Egg	1st	2nd	3rd	Total
C1-2018	12-Jul	0.72	0.61	1.43	**NA**	0.14
C2-2018	15-Jul	1.46	0.43	0.64	1.24	0.17
C3-2018	18-Jul	**2.00**	0.47	0.07	1.28	0.59
C4-2018	22-Jul	0.46	0.88	1.53	0.72	1.71
C5-2018	31-Jul	0.29	1.13	1.00	0.39	1.12
C1-2019	23-Jul	**NA**	1.62	0.94	0.07	**2.50**
C2-2019	25-Jul	**NA**	1.50	0.69	0.61	1.73
C3-2019	27-Jul	**NA**	1.55	0.73	0.65	0.94
C4-2019	29-Jul	**NA**	**1.99**	1.21	0.10	**2.24**
C5-2019	6-Aug	1.07	0.34	1.17	1.24	1.38
C6-2019	15-Aug	1.26	0.86	0.18	0.4	0.65
C7-2019	19-Aug	1.30	0.52	0.46	1.35	0.66
C1-2020	14-Jul	0.59	0.29	1.5	**NA**	1.42
C2-2020	15-Jul	0.53	1.21	0.19	**NA**	**2.77**
C3-2020	17-Jul	0.53	0.62	0.87	1.95	1.75
C4-2020	19-Jul	0.09	1.50	0.83	0.30	**2.37**
CN-2021	12-Jul	1.20	0.49	0.85	1.81	0.16
CS-2021	12-Jul	0.18	0.75	0.49	1.61	0.63



	C5-2019		CN-2021	
Instar	Dissection	Observed	Dissection	Observed
Egg	61	30	4	19
1st	14	92	20	224
2nd	8	63	12	228
3rd	5	26	7	121

Figure 2.1. Graphs comparing the predicted vs observed pupal emergence for two cohorts.

The relative number of early (egg or first) or late (second or third) instars affects the total observed pupal emergence. Predictions of pupal emergence were weighted based on the relative numbers of life stages. Cohort CN-2021 had a relatively even distribution of life stages observed in dissections and the weighted mean emergence was 9.4 days predicted and 9.2 days observed. By comparison cohort C5-2019 had a higher proportion of eggs than any other life stage, and the weighted mean emergence was shifted later to 11.9 days predicted and 10.2 days observed. The discrepancy between predicted and observed could be a result of early instar mortality before pupation. The adjacent table shows the instars that were observed in dissections for each cohort in the figure.

Chapter 3: Effect of rearing temperature on natural mortality of blueberry maggot larvae, *Rhagoletis mendax* (Curran)

3.1. Abstract

Pesticide drift, blueberry variety, co-infestation by spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura), "low" berry quality, hand-picking, and rearing temperature were identified as possible variables contributing to natural mortality of blueberry maggot (BBM), *Rhagoletis mendax* (Curran), which was found to be $60 \pm 26\%$ ($\bar{x} \pm s$) from blueberries collected in Southwest Michigan and reared at 26.6 °C and 80% RH in 2018, 2019, and 2020. The effect of hand-picking and rearing at constant temperature on natural mortality was isolated by utilizing high-quality Bluecrop variety berries from four non-treated rows enclosed in netting that precluded SWD. Blueberries were hand-picked from the netted rows over the span of a single day and reared at a constant temperature of 26.6 or 21.1 °C and rearing efficiency, a proxy for natural mortality, was compared with the efficiency from bagged, intact (i.e. non-picked) blueberries in clusters subject to ambient temperatures. Incubated blueberries had numerically greater rearing efficiency than those bagged at ambient temperatures, however there was no statistical difference, indicating that picking and incubating berries had minimal, if any, effect on natural mortality.

3.2. Introduction

Blueberry maggot (BBM), *Rhagoletis mendax* (Curran), in field-infested highbush blueberries, *Vaccinium corymbosum*, were collected from two locations in southwest Michigan and incubated at 26.6 °C and 80% RH (Chapter 2). Rearing efficiency, r_E, the percentage of blueberries reared that yielded a BBM pupa, and infestation rate, r_i, the percentage of blueberries dissected containing a BBM egg or larva, were calculated for each group of blueberries collected. r_E was observed to be 11 to 90% less than r_i, and the difference was attributed to natural mortality, calculated as $(1 - r_E/r_i) * 100\ \%$. Natural mortality is the mortality expected in any insect population and is discussed here as the mortality of BBM larvae.[69]

Several variables, occurring prior to blueberry collection, could have contributed to natural mortality with the effect of spotted wing drosophila (SWD, *Drosophila suzukii* (Matsumura)) co-infestation and fruit quality discussed in Section 2.3.2.3. Pesticide drift and differences in blueberry varieties, not discussed in Section 2.3.2.3, are unlikely to have contributed to BBM natural mortality, as blueberries from the Southwest Michigan Research and Extension Center (SWMREC) were greater than 20 m from the nearest pesticide sprays, and blueberries collected from Bangor, MI were protected from pesticide drift by a barrier of dense forest. Additionally, differences in the blueberry varieties collected (Bluecrop, Jersey, and Elliot) have similar compositions of citric, malic, succinic, and quinic acids.[70] Other differences between blueberry varieties, not yet studied, may affect BBM host suitability but are outside the scope of this study.

Blueberry maggot are poikilotherms, and the development of immature BBM is dependent on temperatures.[71] The optimum temperature minimizes mortality, aids development, or combinations thereof.[61,72] Natural mortality of BBM across the egg through pupal stage when reared at 26.6 or 21.1 °C was compared to bagged, intact blueberry clusters subject to ambient temperatures by using r_E as a comparative proxy.

3.3. Materials and methods

3.3.1. Blueberry infestation

A total of 96 Bluecrop variety blueberry bushes, ca. 10 years old, were planted in four adjacent, parallel rows at SWMREC, 24 bushes per row. These bushes received natural pressure from BBM and SWD in years prior to 2019. In June of 2019, prior to the emergence of BBM and SWD, netting was installed to cover two adjacent rows, 48 bushes per net. Several hundred BBM pupae, field-collected from 2018, were placed within each of the two nettings in open petri dishes on elevated platforms and provided shelter from rain in each net. It should be noted that the winter of 2017 was atypically cold and without snow, ultimately resulting in a relatively reduced SWD population across Southwest Michigan in 2018. Berries collected from the nettings in 2019 were nearly exclusively infested with BBM relative to SWD. Seeding of the netted plantings occurred again in June 2020 and June 2021, with consistently increasing infestation of BBM and suppression of SWD.

3.3.2. Isolation of intact blueberry clusters

In 2021, Michigan State University's Enviroweather webtool predicted that the first flight of BBM would occur on June 18th (www.enviroweather.msu.edu). The first flight of female BBM require ca. 10 days to mature, mate, and begin laying eggs. Previous experience, Chapter 2, indicates that an additional ca. 14 days are required for eggs to develop through third instar larvae. Accordingly, July 12th was identified as the earliest opportunity to collect blueberries containing all life stages. Inspection of the seeded pupal trays on ca. July 4th revealed that nearly all of the pupal casings were empty. Accordingly, the experiments planned for July 12th would yield blueberries infested with BBM originating from prior seasons, to include those adults collected in 2020.

On July 12^{th}, 30 blueberry clusters were identified and selected in each of the two nettings based on the number and ripeness of the blueberries. A 6-foot safety fence stake (Model No. H-4637, Uline) was hammered vertically into the soil so that the cluster was oriented ca. 3 cm away from its face (Figure 3.2). A 32-oz plastic deli container (US Plastic) with two vertical slits, ca. 4 cm apart and centered on one side, was positioned ca. 10 cm under the cluster and flush with the face of the stake. A cable tie was then woven through the slits of the 32-oz container and around the stake to secure its position under the cluster. An 11- x 14-in. organza gift bag (Model No. S-13170, Uline), containing a 48-oz plastic deli container (US Plastic) with 2 – 5 cm of play sand (Premium Play Sand, Quickrete®), was opened and positioned with the blueberry cluster inside the bag and over the sand while maintaining an upright orientation of the 48-oz container. The drawstrings of the organza bag were then drawn tight over the branch supporting the cluster and care was taken to avoid crushing any foliage. The bag, the 48-oz container, and the cluster were then placed into and completely supported by the 32-oz container such that no additional strain was induced on the blueberry branch. The number of ripe blueberries was recorded before being enclosed, and any blueberries that fell off were maintained with the cluster for the duration of the experiment. Ambient temperatures in the field were recorded using data from Michigan State University's Enviroweather website for SWMREC from July 12, 2021 to August 2, 2021.

3.3.3. Fruit collection

Simultaneous to the isolation of berry clusters on July 12^{th}, blueberries were selected for ripeness, handpicked from each of the two nettings into 6 oz. clamshells (Mid-Lantic Labeling and Packaging), and labeled respective to the netting where they were picked (North or South). Approximately 2250 blueberries were collected in the clamshells from each netting. Respective to each netting, two clamshells were selected and opened, the number of blueberries contained were counted, and all blueberries were transferred to a single rearing container (Section 2.3.2.2). Half of the rearing containers from each netting were incubated at 26.6 °C, and half at 21.1 °C. Temperatures in incubators were monitored with HOBO data loggers (HOBOware version 2.7). An additional ca. 75 blueberries were randomly selected, using the same criterion as above, from each netting for dissections.

3.3.4. Rearing containers

See Section 2.3.2.2 for details concerning rearing containers.

3.3.5. Pupae collection

See Section 2.3.2.4. for details concerning pupae collection from rearing containers. Pupae were collected from clusters by opening the bag around each cluster and replacing the 48-oz containers with new containers of fresh sand before re-securing the bag around the branch. Pupae were collected from removed containers using the same method as rearing containers (Section 2.3.2.4), and the number of pupae was recorded respective to each cluster. Calculations of r_E were conducted per rearing container or blueberry cluster as the number of pupae observed divided by the number of blueberries in the container or blueberry cluster. The grand mean rearing efficiency ($\bar{r}_E$) was calculated across all containers or blueberry clusters in a group (i.e. net or temperature) with a standard deviation ($\pm s$), propagated using standard methods.[56]

3.3.6. Dissections

Initial infestation rates, r_i, and initial BBM life stage distributions were determined as aided by microscope dissections of ca. 75 blueberries per net. See Section 2.3.2.3 for more details.

3.3.7. Statistics

Single-factor ANOVA and post-hoc Tukey's multiple means comparisons were conducted using R and R Studio.[73,74]

3.4. Results and discussion

3.4.1. Initial infestation and natural mortality

Initial dissections of 75 and 76 blueberries from each net, North and South respectively, indicated that all life stages were present with higher proportions of first and second instars. Natural mortality was estimated by comparing r_i to $\bar{r}_E$ from rearing containers and blueberry clusters respective to each net. For the North net, r_i was 61%, $\bar{r}_E$ was 54 $\pm$ 17% ($\bar{r}_E \pm s$), and percent natural mortality was 11%. $\bar{r}_E$ was consistent for each rearing temperature in the North net (Table 3.1), however in the South net, r_i was 28%, $\bar{r}_E$ was 43 $\pm$ 16% ($\bar{r}_E \pm s$), and percent natural mortality was -54%. Blueberries from the South net reared at 26.6 and 21.1 °C had $\bar{r}_E$ values of 53 $\pm$ 8% and 50 $\pm$ 14% ($\bar{r}_E \pm s$), respectively, while blueberry clusters had a $\bar{r}_E$ of 33 $\pm$ 15% ($\bar{r}_E \pm s$). At all three rearing temperatures, more BBM were reared from the South net than predicted by r_i, indicating that dissections did not represent the population r_i. Rather than use percent natural mortality calculations, r_E was used as a comparative proxy for natural mortality based on the assumption that r_i would be the same for blueberries collected or isolated simultaneously from the same population, therefore any difference in r_E would be a result of mortality caused by rearing temperature.

3.4.2. Rearing efficiency

Incubator temperatures were recorded as 26.5 ± 0.6 °C and 22.0 ± 1.2 °C ($\bar{x} \pm s$), and the mean ambient temperature at SWMREC was 22.1 ± 4.0 °C ($\bar{x} \pm s$) with extremes of 11.3 and

31.3 °C. The mean daily minimum and maximum temperatures were 17.5 ± 2.8 and 26.3 ± 3.1 °C ($\bar{x} \pm s$), respectively. Blueberries incubated at constant temperatures of 26.6 and 21.1 °C had each had higher $\bar{r}_E$ values of 52% $\pm$ 8% and 55% $\pm$ 12% ($\bar{r}_E \pm s$) compared to the $\bar{r}_E$ at ambient temperature of 43% $\pm$ 22% ($\bar{r}_E \pm s$) (Table 3.1). However, a single-factor ANOVA did not reject the null hypothesis ($F_{2,59} = 3.11; P = 0.0522;\ \alpha = 0.05$), but only by a slim margin. Results of the single-factor ANOVA indicate the observed differences are unlikely to represent a difference in the "true" population means, and that BBM natural mortality at constant temperatures of 26.6 and 21.1 °C approximates natural rearing.

Any observed difference in $\bar{r}_E$, and thereby BBM natural mortality, could be a result of any uncontrolled variable at ambient conditions (rain events, humidity, temperature variations, plant health, etc). Developmental-temperature thresholds for immature BBM larvae have not been studied, but the lower developmental-temperature threshold used in degree-day models to predict the development of BBM pupae is 10 °C.[75] If the developmental-temperature threshold for immature BBM larvae and pupae are similar, multiple nights near 10 °C could increase immature BBM mortality. Constant temperature might decrease BBM natural mortality by maintaining a constant level of activity rather than approaching inactivity at temperature extremes. Regardless of the exact cause, variation in developmental time is expected in natural populations from year to year.

3.4.3. Developmental time and variance

Developmental time was calculated for each rearing container or blueberry cluster as the weighted mean and standard deviation ($\bar{x}_{pupa} \pm s_{pupa}$) (equations 2.1 and 2.2) duration in days from blueberry collection or isolation to the observation of pupae. The grand mean and standard deviation ($\bar{x}_{GM} \pm s_{GM}$) were then calculated as the mean and standard deviation of $\bar{x}_{pupa}$ across all rearing containers or blueberry clusters each temperature group. As expected, $\bar{x}_{GM}$ was lowest for blueberries held at 26.5 $\pm$ 0.6 °C ($\bar{x} \pm s$) at 9.27 $\pm$ 0.50 days ($\bar{x}_{GM} \pm s_{GM}$) followed by blueberries held at 22.0 $\pm$ 1.2 °C ($\bar{x} \pm s$) at 11.63 $\pm$ 0.70 days ($\bar{x}_{GM} \pm s_{GM}$). The ambient temperature in the field, 22.1 $\pm$ 4.0 °C ($\bar{x} \pm s$), was very similar to the incubator set to 21.1 °C, however $\bar{x}_{GM}$ for ambient temperatures was longer at 12.92 $\pm$ 2.04 ($\bar{x}_{GM} \pm s_{GM}$) days. A single-factor ANOVA rejected the null hypothesis that $\bar{x}_{GM}$ at each temperature was the same ($F_{2,59} = 29.97; P = 1.04 \times 10^{-9}$; $\alpha = 0.05$), and a post-hoc Tukey's multiple means comparison indicated statistically significant differences between the $\bar{x}_{GM}$ for each temperature.

The mean standard deviation of developmental time, $\bar{s}_{GM}$, for each temperature grouping was calculated to compare the effect of temperature on developmental variability. A single-factor ANOVA rejected the null hypothesis that $\bar{s}_{GM}$ was the same between temperature groupings ($F_{2,58} = 8.977; P = 4.01 \times 10^{-4}$; $\alpha = 0.05$). A post-hoc Tukey's multiple means comparison indicated a statistical difference between 26.6 °C and ambient temperatures but not between 26.6 °C and 21.1 °C or 21.1 °C and ambient temperatures. The relationship between temperature and developmental variability is not well generalized, however for BBM constant rearing temperature appeared to decrease developmental variability.[72] Examination of Figure 3.1 shows that the initial pupal emergence curves for 21.1 °C and ambient temperatures were the same, but at ambient temperatures, there were more BBM with longer developmental times leading to greater developmental variability.

3.5. Conclusion

Removing infested blueberries from the bush had no negative effect on BBM natural mortality, and there was some weak evidence to indicate improved r_E at constant temperatures of 26.6 and 21.1 °C. More replications would be required to confirm the observed improvement over ambient temperatures. Rearing at 26.6 °C decreased the developmental time by ca. 2 days and ca. 4 days compared to 21.1 °C and ambient temperatures, respectively, and constant temperature also reduced developmental variance compared to ambient temperatures. Based on these results, 26.6 °C was the best temperature for the retrospective model, as developmental time was the shortest without increasing developmental variability (Section 2.4.5). Additionally, the consistent r_E across all conditions show that the rearing method had no impact on BBM mortality, and any mortality observed in fumigation experiments, relative to non-treated controls, was the result of fumigant toxicity alone.

Table 3.1. Blueberry maggot rearing results

Blueberries from the Southwest Michigan and Research Extension Center were collected from or isolated as intact clusters in two adjacent insect exclusion nets. Mean rearing efficiency, $\bar{r}_E \pm s$, was calculated as the percentage of blueberries that produced a pupa for all containers or clusters in each temperature and net group.

Table 3.1

	Blueberries			Pupae		
Origin/Location	26.6 °C	21.1 °C	Ambient	26.6 °C	21.1 °C	Ambient
North net	1125	1125	360	577	656	181
South net	1125	1125	286	594	574	90
Total	2250	2250	646	1171	1230	271

Rearing efficiency (r_E)

	26.6 °C		21.1 °C		Ambient	
Origin/Location	$\bar{r}_E$	s	$\bar{r}_E$	s	$\bar{r}_E$	s
North net	52%	8%	59%	7%	53%	24%
South net	53%	8%	50%	14%	33%	15%
Total	52%	8%	55%	12%	43%	22%

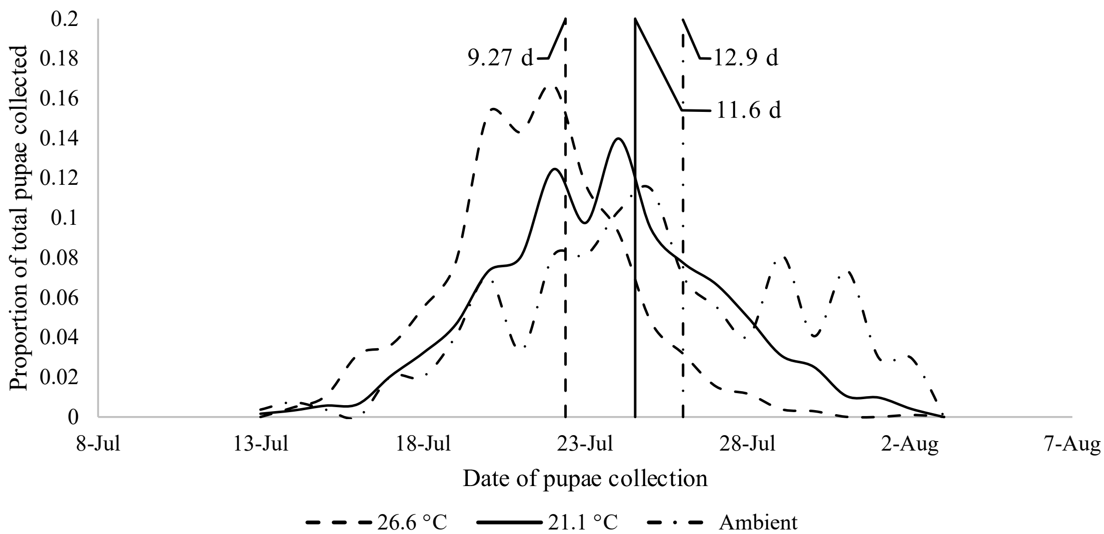


Figure 3.1. Mean blueberry maggot pupae emergence

The weighted mean blueberry maggot (BBM) pupal emergence was calculated for each set of rearing temperatures (vertical lines) to compare the effect of temperature on BBM development time. Pupal emergence was recorded daily for 21 days. The proportion of total pupal emergence is plotted to compare the pupal emergence distributions of the different rearing methods.

Figure 3.2. Example of blueberry cluster isolation.

Intact blueberry clusters were isolated using 11 x 14-inch organza bags, and pupae were collected in sand held below the clusters. The bag and sand container were supported by a second plastic cup attached to a 6’ tall security fence post.

Chapter 4: Methyl bromide fumigation of blueberry maggot, *Rhagoletis mendax* (Curran), and integration with a systems-based approach

4.1. Abstract

Methyl bromide (MB) chamber fumigations were evaluated as a part of a systems-based approach for control of blueberry maggot (BBM), *Rhagoletis mendax* (Curran), in fresh blueberries from the Eastern US. Blueberries naturally infested with BBM were gathered from plantings not subject to control measures. Infested blueberries were fumigated with 64 mgL^{-1} MB for 2 h at pulp temperature, T, of 10.6 ºC. These fumigations yielded observed concentration × time exposures that ranged from 92 to 116 mgL^{-1} h with a mean of 109 mgL^{-1} h, consistent with the fumigation schedule proposed by USDA-ARS to control spotted wing drosophila, *Drosophila suzukii* (Matsumura) (load ≤ 55%, 64 mgL^{-1} MB, 2 h, $T \geq 10.6$°C). Treatment efficacy was diagnosed by the percentage of survivors entering pupation from fumigated blueberries relative to that from non-fumigated controls. Fumigations resulted in 2 survivors from 3928 ± 89 treated ($\bar{x} \pm s$), 99.84% mortality (probit 7.95, 95% confidence level). MB fumigation efficacy was combined with insecticide application efficacy to yield a joint probability of BBM control of 99.9974% efficacy or probit 9.05 at the 95% confidence level.

4.2. Introduction

While methyl bromide (MB) is being phased out due to regulations to protect the ozone layer, it still remains a valuable tool for gaining market access.[29] Methyl bromide has many of the ideal characteristics for a postharvest fumigant as it is volatile, toxic to insects, and penetrates into commodities with minimal residues (Table 1.1). From a regulatory standpoint, MB is also one of only a few fumigants that are internationally accepted, and for fresh blueberries, it is already in use for control of spotted wing drosophila (SWD, *Drosophila suzukii* (Matsumura)), which also infests ripe blueberries. [19,76] Recently, USDA-ARS research demonstrated the complete control of SWD when 64 mgL^{-1} MB was applied for 2 h at pulp temperature, T, ≥ 10.6°C. [77] The concentration (C) × time (t), Ct, products observed across the SWD trials ranged from 100 to 111 mgL^{-1} h (mean = 106 mgL^{-1} h), relative to the theoretical Ct, $(Ct)_{th}$, 128 mgL^{-1} h, because ca. 30% of the applied MB was sorbed by the packaged blueberries.[77]

Methyl bromide has some proven efficacy against BBM as shown by Roth and Richardson who treated 333 BBM eggs and larvae with >32 mgL^{-1} MB for 2 h at $T \geq 10$°C.[78] This MB fumigation schedule was adopted by Washington and California, both BBM-free, to maintain BBM quarantines for imported fresh blueberries. Zero survivors in 333 BBM treated is equal to a mortality of 99.11% (probit 7.37, 95% confidence level (CL)),[79] but National Plant Protection Organizations (NPPO) require higher statistical metrics, with probit 9 as the established standard.[47]

The efficacy of the proposed SWD fumigation schedule (load ≤ 55%, 64 mgL^{-1} MB, 2 h, $T \geq$ 10.6°C) toward BBM was quantified, and additional fumigations were also conducted with $(Ct)_{th}$'s of 40 and 80 mgL^{-1} h as a means of identifying the relative tolerance of BBM life stages, potentially more obvious at sub-lethal exposures. We detail below the MB fumigation conditions required to disinfest fresh blueberries of SWD and BBM when combined with existing integrated pest management (IPM) strategies as a systems-based approach.

4.3. Materials and methods

4.3.1. Fruit collection – fumigation

Blueberries were collected from a planting near Bangor, MI last operated commercially in the early 2000's. All blueberries collected on the same day were labeled by date, a cohort. In 2018, blueberry cohorts were collected from Bangor, MI on July 12, 18, and 22. In 2020, one blueberry cohort was collected from Bangor, MI on July 16. Blueberries were picked into buckets from throughout the field and then evenly distributed by hand from each of the buckets to fill 6 oz. clamshells (Mid-Lantic Labeling and Packaging, Hammond, NJ).

Specific to each collection date, three clamshells were randomly selected and reserved for dissection to reference the chronology of development in the collection cohort relative to the stage-frequency metrics of the previously established life history table (Chapter 2). Clamshells were then loaded to completely fill two 411-L wine coolers (Summit Appliance, Bronx, NY) set to the fumigation temperature of 10.6°C. Remaining clamshells, generally numbering 10 to 20% of those loaded into the coolers, were emptied and the blueberry contents of two clamshells were transferred into a rearing container (vide infra). All rearing containers were then placed into an incubator at 26.6 °C and 80% RH. These remaining blueberries represented the controls for the MB fumigations.

4.3.2. Fruit collection – pesticide efficacy

In 2018, 2019, and 2020, BBM infestations at two unmanaged blueberry fields (Site A and Site B) were monitored for the presence of BBM by collecting blueberries and incubating them under BBM rearing conditions (vide supra). In 2020, five managed commercial blueberry fields were also monitored for BBM infestation using the same blueberry collection and rearing method above (Table 4.1).

4.3.3. Rearing containers

See Section 2.3.2.2 for details concerning rearing containers.

4.3.4. Chemicals and chemical analysis

A 5-lb cylinder of compressed MB, Meth-o-gas 100, was obtained from Cardinal Professional Products (Woodland, CA). Methyl bromide headspace concentration, [MB], was measured using gas chromatography with a thermal conductivity detector (GC-TCD). Detector response and retention indices were determined each day in calibration studies by diluting known volumes of MB into a volumetric gas vessel. Retention time, as determined prior to each experiment, was used for chemical verification. The integral of peak area, referenced relative to linear least squares analysis of a 5-point concentration–detector response curve, was used to determine [MB] concentration. Analyses were conducted with a Varian CP- 4900 using a gas sampling port (110 °C) with a 1-mL sample loop and a capillary 10 m x 0.25 mm PoraPLOT Q held at 130 °C for 1.2 min receiving 3.74 ml min^{-1} He carrier flow, 20.0 psi.

The limit of blank (LOB) and limit of detection (LOD) were determined from instrument signal response according to the method of Armbruster and Pry.[80] The Varian CP4900, described above, was inaccessible, and an SRI Instruments 8610C with TCD was used as a proxy. Samples were introduced through a gas sampling port with 1.0-mL sample loop and a packed 1.8-m x 3.2-mm HayeSep-D column held at 180°C for 2.5 minutes receiving 30 ml min^{-1} He carrier flow, 25.0 psi. The mean signal background in blank samples ($\bar{x}_{blank}$) was calculated with standard deviation (s_{blank}) and propagated according to standard methods.[56] The LOB was calculated as $\bar{x}_{blank}$ +

$1.645(s_{blank})$. The LOD was then calculated as LOB $+1.645(s_{LC})$, where s_{LC} is the standard deviation of peak detector signal from "low concentration" samples.

4.3.5. Exploratory fumigations

Fumigant-induced mortality of BMM egg and larval life stages was evaluated following laboratory-scale exploratory fumigations conducted in a matched set of three modified Labonco® (Labconco Corporation, Kansas City, MO) 28.32-L vacuum chambers housed in coolers set to a fumigation temperature of 10.6°C.[81] Six clamshells of naturally infested blueberries were removed from the wine coolers, having been stored at 10.6°C for at least 24 hours, and placed into each of the three chambers. After the chambers were loaded, the circulation fans were turned on before clamp-sealing the doors in preparation for treatment. Circulation in the chambers was achieved using 120-mm fans powered by 6-V flashlight batteries (Harbor Freight, Camarillo, CA). Batteries were replaced when their measured voltage dropped below 5 V. A vacuum of ca. 254 mmHg was established in each chamber. Gas-tight super-syringes were filled with a pre-determined volume of MB and fitted to a LuerLok ® sampling valve, which was subsequently opened so that MB was steadily drawn into the chamber. After the addition of MB from the final syringe, the syringe was removed from the valve and normal atmospheric pressure (NAP) was re-established in each chamber before the valve was closed; this marked the start of the fumigation and the beginning of the exposure period. Gas samples (10 mL) were taken from the chamber headspace through the sampling valve using a B-D® 30-mL gas-tight syringe and [MB] was quantified with GC-TCD, as described above, at temporal intervals of time, *t*, corresponding to 5 (initial), 60 (mid-point), and 120 min (final) (e.g., $[MB]_0 \approx [MB]_{t=5}$, $[MB]_{t=30}$, etc.). Fumigant exposures were expressed as *Ct* products (mgL^{-1} h) and calculated by the method of Monro.[28] After the exposure period, chambers were moved to a fume hood, a vacuum system was attached and activated for a 30-min aeration period, and valves were opened to atmosphere to re-establish NAP. Chamber lids were opened, the treated blueberries were collected and transferred into rearing containers as above. Rearing containers were then placed in an incubator maintained at 26.6°C and 80% RH.

4.3.6. Regional blueberry maggot infestation

Single-factor ANOVA was used to compare BBM pupae reared from each location-year combination for Site A in 2018, 2019, 2020 and Site B 2020 (Table 4.2) to determine if BBM infestation in the region was similar at the 95% CL.[73,74] The ANOVA was significant and results were further analyzed using Tukey's multiple means comparison (α = 0.05) (Table 4.3).[73,74] Statistical analyses were conducted using R and R Studio.[73,74]

4.3.7. Natural infestation

Record was kept of the cumulative number of pupae that emerged from each blueberry collected in 2020 from unmanaged blueberry fields at Site A and Site B. Rearing efficiency, r_E, was calculated for each container from Site A and Site B as the number of emerged pupae divided by the number of blueberries in a container. The grand mean rearing efficiency ($\bar{r}_E$) was calculated across the containers with a standard deviation ($\pm s_{GM}$) calculated, via standard methods,[56] by propagating the respective standard deviations of the containers. The number of specimens ($n \pm s$) that were present in a collection from each managed commercial field was estimated by multiplying the number of infested berries by $\bar{r}_E \pm s_{GM}$ (Table 4.4).

The number of BBM present in fumigation trials was similarly estimated, where $\bar{r}_E \pm s_{GM}$ was calculated across the non-treated control containers respective to each collection cohort. The number of blueberries treated in separate fumigations was then multiplied by $\bar{r}_E \pm s_{GM}$ to yield the number of BBM in each fumigation trial with a standard deviation. The total number of specimens treated in each exposure range was calculated as the summation of separate fumigations, and error was propagated via standard methods.[56]

4.3.8. Mortality assessment

With respect to recording mortalities of BBM in treated blueberries, pupae found when sifting sand from a rearing container were recorded as survivors (v) (Section 2.3.2.4). Third instars found in the sand were only counted as survivors if they exhibited prodding induced motion. For a specific fumigation trial, mortality was calculated as the fractional percentage of $(n - v)\ n^{-1} x\ 100$. The mortality percentage across all trials was expressed as a function of the number of specimens treated via probit analysis of Finney[69,82] at the 95% CL, as further derived in Couey and Chew as well as Liquido and Griffin.[79,83]

4.3.9. Most methyl bromide-tolerant life stage

Relative life stage tolerance to MB fumigation was assessed using multiple, pairwise comparisons ($p < 0.05$) (Table 4.5) (See Table 4.6 for the number of survivors for each life stage and Table 4.7 for the number of each life stage treated). The Bonferroni correction was used to adjust p values for multiple comparisons. There was no statistical evidence for a most MB tolerant life stage.

4.3.10. Systems-based approach

In the case where one event, E_1, has no effect on the probability of the other(s), the joint probability of BBM control associated with multiple treatment events, $P(E_1 + E_2 + E_n)$, can be calculated from the multiplication of the simple probability of each event:[82]

$$P(E_1 + E_2 + E_n) = 1 - (1 - P(E_1))(1 - P(E_2))(1 - P(E_n))$$

(eq. 4.1)

Given equation 4.1, the special multiplication rule for independent events, the probability of BBM control following the joint occurrence of two or more treatment events can be calculated for numerous scenarios directly applicable to preharvest and postharvest pest management procedures used in Michigan.

4.4. Results and discussion

4.4.1. GC-TCD analysis

Methyl bromide concentrations used for postharvest fumigation do not approach the limits of quantification for GC-TCD as the lowest concentration quantified in the MB fumigation trials was 7.43 mgL^{-1}. Detector signal from blank samples ($n = 18$) from 1 to 2 min, corresponding with the retention time of MB ($t_r = 1.6$ min), were used to calculate $\bar{x}_{blank} \pm s_{blank}$ as 3.60 ± 3.37 mV, and the LOB was 8.10 mV. The LOD was 65.79 mV, and triplicate standards of 7.34 mgL^{-1} MB produced a mean peak detector signal of 672 ± 16 mV ($\bar{x} \pm s$), 10-fold greater than the LOD (Figure 4.1).[80]

A matched set of five modified 28.32-L fumigation chambers with circulation fans were dosed to yield [MB] of 7.34 mgL^{-1} after 5 minutes. The integral of peak area, referenced relative to linear least squares analysis of a 5-point concentration–detector response curve (Figure 4.2), was used to determine the mean [MB] concentration in the chambers as 6.45 ± 0.20 mgL^{-1} ($\bar{x} \pm s$) that yielded 88% of the target concentration, 7.34 mgL^{-1} (Figure 4.3).

4.4.2. Regional blueberry maggot infestation

The single-factor ANOVA to compare BBM $\bar{r}_E$ at Site A in 2018, 2019, 2020, and Site B in 2020 was significant ($F_{3,14} = 6.349$, Pr (>F) 0.0061), and the null hypothesis rejected (Table 4.2). A post-hoc Tukey's multiple means comparison ($\alpha = 0.05$)[73,74] indicated significantly different infestation for 2018 Site A compared to 2019 Site A, 2020 Site A and 2020 Site B. Different BBM infestation levels in 2018 are likely due to late emergence of SWD. No difference was indicated between 2020 Site A and 2020 Site B suggesting that regional BBM infestations are similar (Table 4.3).

4.4.3. Preharvest insecticide efficacy

In the managed commercial fields, 12,074 blueberries were collected and held at rearing conditions until any BBM present had opportunity to pupate (Table 4.4). An estimated 1317 pupae were present and only 3 BBM pupae were observed resulting in a 99.41% mortality (probit 7.52, 95% CL) (Table 4.8).

4.4.4. Postharvest methyl bromide efficacy

Based on observations made during dissections, we had hypothesized that the early second instars would be the most tolerant to MB fumigation. Second instars were observed at the center of mostly intact blueberries, where the flesh of the blueberry might act as a barrier to MB. Eggs and first instars were observed near the surface of the blueberry and third instars in hollow blueberries. No life stage was observed to be significantly more tolerant than the others (Table 4.5). The survivors observed at the highest *Ct* range (108.9 ± 7.0 mgL^{-1} h) were eggs, however there were still only two surviving eggs from an estimated 588 eggs treated, which was not statistically significant (Table 4.5). In the absence of a most MB tolerant life stage, the total number of immature life stages treated could be pooled.

An estimated total of 11,890 immature BBM were treated across the three targeted $(Ct)_{th}$'s of 40, 80, and 120 mgL^{-1} h (Table 4.9). Table 4.10 shows the probit calculations including $\pm 2s$. An estimated 3928 BBM treated with MB at 10.6 °C with a *Ct* of 108.9 ± 7.0 mgL^{-1} h. Only two survivors were observed resulting in a 99.84% mortality (probit 7.85) at the 95% CL as calculated by Couey and Chew.[79] A mean *Ct* of 108.9 mgL^{-1} h is consistent with the MB schedule proposed by USDA-ARS for the control of SWD (load ≤ 55%, 64 mgL^{-1} MB, 2 h, $T \geq 10.6$°C).[77] "Natural" mortality in control specimens was assumed to be equal to that in fumigation trials.

4.4.5. Methyl bromide sorption kinetics

The possibility of a standalone MB fumigation would be limited by the maximum label parameters for MB fumigation and the MB sorption kinetics in commercial fresh blueberry fumigations. The maximum label rate for MB fumigation of fresh blueberries is 3 h at 64 mgL^{-1} (US Environmental Protection Agency registration number 8536-15). For commercial fumigations with large chamber loads, the loss of headspace fumigant concentrations due to sorption of the fumigant into the load (fruit and packaging) can be significant, as postharvest fumigation schedules are developed with a minimum required headspace concentration and duration to achieve target mortality.[27] Because methyl bromide fumigations are generally short, any sorption can be described as a reversible, mass transfer-limited diffusion, as there is not sufficient time to reach the equilibrium air-substrate distribution of MB.[84] Surface-specific parameters can be calculated for various fruits and packaging materials; however, this is unnecessary.[85,86] The rate that [MB] is depleted for a packaged load can be determined empirically for standardized packaging, as the loss of [MB] is dominated by the sorption rate into the commodity. The rate of [MB] loss can be described generically using the first-order differential rate equation:

$$-\frac{d[MB]}{dt} = -k_{OBS}[MB]$$

(eq. 4.2)

Where k_{OBS} (h^{-1}) is the observable rate constant of [MB] loss from chamber headspace and represents the combined effect of sorption into the commodity and packaging. The rate of sorption is proportional to the surface area of the commodity and packaging, which changes as a fixed ratio of the quantity of commodity per standardized package. The relationship between k_{OBS} and the specific rate constant of a packaged commodity, k_{SPT}, can be conveniently described by the following equation:

$$k_{OBS} = \left(\frac{V_{PL}}{V_{chamber}}\right)\left(\frac{SA}{V_{carton}}\right)k_{SPT}$$

(eq. 4.3)

Where V_{PL} is the volume of the packaged load, $V_{chamber}$ is the volume of the fumigation chamber, SA is the total surface area of fruit in the carton, and V_{carton} is the volume of a carton or other standardized container (i.e., bin, tray, clamshell, etc.). Using these quantities, equation 4.3 establishes an empirical relationship between k_{OBS} and k_{SPT}.[85–87] By understanding the kinetics of MB sorption, and applying the above model, load, dose, and time can be modulated based on specific packaging to achieve the level of mortality required to control BBM.[85–87]

Data from previous work by Tebbets and Walse to confirm a MB fumigation for SWD in fresh blueberries can be used to calculate k_{SPT} in an export scenario.[77] Plastic clamshells of blueberries in cardboard trays were treated in 241.9-L steel chambers at 54.6% load to yield a k_{SPT} of $3.72 * 10^{-4} \pm 0.90 * 10^{-4}$ m h^{-1} ($\bar{x} \pm s$) at 10.0 °C. With regard to a maximum achievable *Ct*, k_{SPT} can be used to calculate a *Ct* of 142.6 ± 9.7 mgL^{-1} ($\bar{x} \pm s$) after a 3-h MB fumigation at 64 mgL^{-1}.[77] More research would be required to determine if a *Ct* of 142.6 ± 9.7 mgL^{-1} ($\bar{x} \pm s$) could provide standalone control of BBM at a probit 9 level, however, increasing fumigation duration would decrease blueberry shelf life and require more MB than a systems-based approach.[88–90]

4.4.6. Systems-based approach evaluation

Systems-based approaches to quarantine security have been defined as "the integration of those pre- and post-harvest practices used in production, harvest, packing and distribution of a commodity that cumulatively meet the requirements of quarantine security" by Jang and Moffitt.[47] The general rule for the multiplication of probabilities, expanded in the seminal works of Finney[82] and Rosenthal[91] on combining results (probabilities) of independent events can be used to quantify the cumulative effect of consecutive pre- and postharvest treatments as events on the "systemic" joint probabilities of control for BBM in fresh blueberries.

For each preharvest and/or postharvest treatment "event" the observed likelihood (expressed as a percentage) of finding a live BBM pupa after treatment, the theoretical percentage of BBM calculated at the 95% CL by the method of Couey and Chew,[79] and the associated probability, $P(E_x)$, are listed in Table 4.11. Also listed are the respective probit values at the 95% CL as calculated according to Liquido and Griffin.[83] For entomological pests, a statistical benchmark of probit 9 is a goal for indexing phytosanitary treatment efficacy.[1,79]

Given the two control events tested in this paper, the solution of equation 4.1 yields a joint probability for BBM control of P = 0.999974 (99.9974% efficacy, probit 9.05) when insecticide treatment is followed consecutively by the application of a methyl bromide postharvest fumigation (Table 4.12).

4.4.7. Systems-based approach integration

Historically, postharvest fumigants have been the preferred method of risk mitigation for exported commodities, as they are reproducible in addition to logistical and operational advantages. Postharvest fumigations have short durations allowing for high throughput, and they are conducted at centralized facilities without opportunity for reinfestation. Continued pressure to eliminate the use of ozone depleting substances like MB, has incentivized NPPO's to consider systems-based approaches. Ultimately, the NPPO of each country will determine what they deem an acceptable mitigation of risk whether by monitoring, insecticide applications, fumigation, or a combination of all three. The Canadian blueberry certification program (D-02-04) is one example of a systems-based approach that outlines a set of guidelines for transportation of blueberries from BBM infested regions in the US and Canada to non-infested regions of Canada. Because pesticide regulations can vary from region to region, the blueberry certification program does not require specific pesticides or application rates. Instead, growers can follow a "calendar spray program", which requires periodic pesticide application, or they can follow an IPM program that outlines specific placement, monitoring, and replacement of yellow "V-shaped" sticky traps baited with ammonium acetate. Canada's blueberry certification IPM program does not require pesticide sprays unless BBM are detected and is ideal for low pest-pressure areas and reducing overall pesticide use. Either the "calendar spray" or IPM programs are suitable for organic production when organic pesticides are used. Regardless of the methods used to suppress BBM populations, fruit from each collection is inspected for infestation that may not have been detected by traps. Under this current program any detection of BBM in inspection results in the rejection of the entire harvest, and the blueberries are limited to sale in BBM-infested regions.

If accepted by other NPPO's, Canada's blueberry certification program could be used as a template for monitoring and treating fresh blueberries without the use of MB when BBM is the primary pest of concern. For Australia and New Zealand, SWD is the primary pest of concern, and MB will still be required for market access, based on our assessment of fields that are already sprayed for BBM and SWD. The specific monitoring methods outlined, combined with sample inspections of ungraded fruit, and then followed by MB fumigation provides layered checks and controls for determining BBM infestation all while making use of in-field pesticides optional.

4.5. Conclusion

Fresh blueberry production has outpaced demand in North America. The market has saturated and both fresh and frozen blueberry prices have plummeted. There is an urgent need to overcome the BBM trade barrier and take advantage of reciprocal markets in the Southern Hemisphere. Markets with high purchasing power in Australia and New Zealand are withholding access to all US blueberry growers based on the implementation of a treatment for BBM. By combining monitoring methods with MB fumigation statistical efficacy for BBM control can achieve probit-9 efficacy required by most NPPO's. The systems-based approach demonstrated above provides an expeditious route towards market access by quantifying the joint effect of preharvest pesticide treatments with the existing postharvest MB treatment for SWD. By utilizing already approved treatments, unnecessary regulatory delays can be circumvented while simultaneously avoiding the use of additional MB that a "standalone" treatment would require to control BBM.

Table 4.1. Blueberry maggot reared from unmanaged blueberry fields.

Blueberries (BB) were collected in cohorts from two unmanaged BB fields in Southwest Michigan and held at a constant rearing temperature of 26.6 °C. The number of BB and blueberry maggot (BBM) pupae was recorded for each cohort.

Table 4.1				
BB collection Date	**Collection site**	**Blueberry variety**	**Sample size (BB)**	**Number of BBM pupae**
7/13/2020	Site A	Jersey	1792	277
7/14/2020	Site A	Jersey	1920	91
7/16/2020	Site A	Jersey	2169	230
7/22/2019	Site A	Jersey	1882	451
7/25/2019	Site A	Jersey	285	48
7/27/2019	Site A	Jersey	678	31
7/29/2019	Site A	Jersey	463	19
7/12/2018	Site A	Jersey	2208	1416
7/15/2018	Site A	Jersey	1891	1032
7/18/2018	Site A	Jersey	1596	820
7/22/2018	Site A	Jersey	1327	253
7/27/2020	Site B	Jersey	100	4
7/27/2020	Site B	Jersey	100	3
7/27/2020	Site B	Jersey	100	2
7/27/2020	Site B	Jersey	100	13
7/27/2020	Site B	Jersey	100	26
8/10/2020	Site B	Jersey	100	41
8/6/2020	Site B	Jersey	100	4

Table 4.2. ANOVA table statistics for blueberry maggot infestations in unmanaged fields.

ANOVA and Tukey multiple comparison of means were used to compare the blueberry maggot infestation per blueberry across years and locations. Only 2018 Site A was significantly different than other sites or years.

Table 4.2					
	Df	Sum Sq	Mean Sq	*F* value	*Pr*(>*F*)
Treatment	3	0.3847	0.1282	6.349	0.0061
Error	14	0.2828	0.0202		

Table 4.3. Tukey multiple comparison of means for BBM infestations in unmanaged fields.

A post-hoc Tukey multiple comparison of means was used to identify the respective differences in infestation across years and locations of the two unmanaged blueberry fields. Only 2018 Site A was in a statistically different grouping.

Table 4.3

year	location	grouping
2020	Site A	a
2019	Site A	a
2018	Site A	b
2020	Site B	a

Table 4.4. Blueberry collections from managed commercial fields.

Blueberries (BB) from five managed commercial farms in Southwest Michigan were collected and incubated at a constant temperature of 26.6 °C. The number of BB and blueberry maggot (BBM) pupae were recorded. The estimated number of BBM pupae along with a standard deviation (*s*) was calculated using the mean infestation rate and standard deviation from the unmanaged commercial fields.

Table 4.4

BB collection date	Collection site	Blueberry variety	Sample size (BB)	Number of BBM pupae	Estimated number of BBM pupae	Standard Deviation (*s*)
7/29/2020	Farm 1	Elliott	250	0	27	19
7/29/2020	Farm 1	Jersey	420	0	46	31
7/29/2020	Farm 1	Bluecrop	357	0	39	27
8/11/2020	Farm 1	Jersey	420	0	46	31
8/11/2020	Farm 1	Elliott	250	0	27	19
8/11/2020	Farm 1	Bluecrop	357	0	39	27
8/7/2020	Farm 1	Jersey	420	0	46	31
8/7/2020	Farm 1	Elliott	250	0	27	19
8/7/2020	Farm 1	Bluecrop	357	0	39	27
7/30/2020	Farm 2	Jersey	420	0	46	31
7/30/2020	Farm 2	Elliott	250	0	27	19
7/30/2020	Farm 2	Bluecrop	357	0	39	27
8/13/2020	Farm 2	Jersey	420	0	46	31
8/13/2020	Farm 2	Bluecrop	357	0	39	27
7/31/2020	Farm 3	Elliott	250	0	27	19
7/31/2020	Farm 3	Bluecrop	357	0	39	27
7/31/2020	Farm 3	Jersey	420	0	46	31
8/6/2020	Farm 3	Elliott	250	0	27	19
8/6/2020	Farm 3	Jersey	420	0	46	31
8/5/2020	Farm 3	Bluecrop	357	0	39	27
8/14/2020	Farm 3	Jersey	420	2	46	31
8/14/2020	Farm 3	Bluecrop	357	0	39	27
8/14/2020	Farm 3	Elliott	250	0	27	19
7/31/2020	Farm 4	Bluecrop	357	0	39	27
7/31/2020	Farm 4	Jersey	420	0	46	31
8/4/2020	Farm 4	Jersey	420	0	46	31

8/4/2020	Farm 4	Bluecrop	357	0	39	27
8/4/2020	Farm 4	Elliott	250	0	27	19
8/11/2020	Farm 4	Bluecrop	357	0	39	27
8/11/2020	Farm 4	Jersey	420	0	46	31
8/11/2020	Farm 4	Elliott	250	0	27	19
8/4/2020	Farm 5	Elliott	250	0	27	19
8/4/2020	Farm 5	Bluecrop	357	1	39	27
8/4/2020	Farm 5	Jersey	420	0	46	31
8/13/2020	Farm 5	Elliott	250	0	27	19

Table 4.5. Pairwise comparison of life stage tolerance.

Relative tolerance of immature blueberry maggot life stages to methyl bromide fumigation was determined by comparing the relative proportion of survivors for each life stage in series of pairwise tests with a Bonferroni adjustment. There was no statistical difference between the tolerance of the life stages at any of the treatment ranges.

Table 4.5

Ct (mgL^{-1} h)		Egg	First	Second
	First	0.37	-	-
108.9 ± 7.0	Second	0.61	1.00	-
	Third	1.00	1.00	1.00
		Egg	First	Second
	First	1.00	-	-
74.8 ± 7.3	Second	1.00	1.00	-
	Third	1.00	1.00	1.00
		Egg	First	Second
	First	0.054	-	-
37.2 ± 2.8	Second	0.069	1.000	-
	Third	1.000	0.427	0.402

P values were adjusted using the Bonferroni method

Table 4.6. Methyl bromide fumigation at 10.6 °C.

Fumigations were conducted at three exposure ranges to determine the tolerance of immature blueberry maggot to methyl bromide fumigations. The exposure range that matches the SWD schedule is separated by a dashed line. The life stages of the survivors were predicted based on temporal pupal emergence and are indicated with single letter abbreviations (E = egg, F = 1st instar larvae, S = 2nd instar larvae, T = 3rd instar larvae). Individual life stages were pooled together based on work indicating no most-tolerant life stage. Mortality probability (*P*(Mortality)) and Total Probit were calculated with the pooled surviving life stages at the 95% confidence level (95% CL).

Table 4.6

Exposure (mgL^{-1} h)	Survivors	Total Treated	*s*	*P*(Mortality) (95% CL)	Total Probit (95% CL)
108.9 ± 7.0[a]	2E	3928	89	0.9984	7.95
74.8 ± 7.3[b]	1F 1S	3944	89	0.9984	7.95
37.2 ± 2.8[c]	6E 2F 1S 4T	4018	92	0.9949	7.57

[a] *targeted* Ct_{th} of 120 mgL^{-1} h, exposure consistent with SWD schedule (load ≤ 55%, 64 mgL^{-1} MB, 2 h, $T \geq 10.6$°C)
[b] *targeted* Ct_{th} of 80 mgL^{-1} h

[c] *targeted Ct_{th}* of 40 mgL^{-1} h

Table 4.7. Number of blueberry maggot treated per life stage.

The relative numbers of life stages treated at each methyl bromide exposure range were estimated based on the retrospective model with standard deviation (s).

Table 4.7

Exposure (mgL^{-1} h)	Egg	s	First	s	Second	s	Third	s
108.9 ± 7.0[a]	588	15	1691	35	1209	28	437	14
74.8 ± 7.3[b]	601	15	1597	34	1191	28	552	17
37.2 ± 2.8[c]	615	15	1616	35	1216	29	569	18

[a] *targeted Ct_{th}* of 120 mgL^{-1} h, exposure consistent with SWD schedule (load ≤ 55%, 64 mgL^{-1} MB, 2 h, $T \geq 10.6$°C)

[b] *targeted Ct_{th}* of 80 mgL^{-1} h

[c] *targeted Ct_{th}* of 40 mgL^{-1} h

Table 4.8. Pesticide treatment efficacy in managed commercial fields.

The number of blueberry maggot (BBM) present at managed commercial fields was estimated from the mean number of BBM per blueberry that was observed from Site A and Site B. The probit level and percent mortality were then calculated based on $\bar{x} \pm 2s$ at the 95% confidence level (95% CL).

Table 4.8

	$-2s$	$\bar{x}$	$+2s$
# BBM estimated	1003	1313	1623
Survivors	3	3	3
Probit (95% CL)	7.42	7.52	7.59
% Mortality (95% CL)	99.23	99.41	99.52

Table 4.9. Methyl bromide fumigation at 10.6 °C: Trial details

Each line lists the fumigation trial details including the theoretical exposure $(Ct)_{th}$, the measured exposures (Ct), the number of blueberries treated, most probable surviving life stage (Surv.) (E= egg, F = 1st instar larvae, S = 2nd instar larvae, T = 3rd instar larvae), estimated number of blueberry maggots treated, date treated, and treatment temperature. Blocks separated by solid lines correspond to the three treatment ranges.

Table 4.9

# BB	Target $(Ct)_{th}$ (mgL^{-1} h)	Ct (mgL^{-1} h)	Surv.	Treated	s	Date	Temp	Location
450	120	116.9		231	28	7/19/2018	10.6 C	Bangor
489	120	116.6		251	30	7/20/2018	10.6 C	Bangor
420	120	114.2		395	23	7/14/2018	10.6 C	Bangor
508	120	113.8		53	7	7/17/2020	10.6 C	Bangor
542	120	113.1		57	7	7/17/2020	10.6 C	Bangor
519	120	112.9		55	7	7/17/2020	10.6 C	Bangor
529	120	112.5		56	7	7/17/2020	10.6 C	Bangor
450	120	111.9		231	28	7/20/2018	10.6 C	Bangor
564	120	111.7		59	7	7/17/2020	10.6 C	Bangor
571	120	111.1		60	7	7/17/2020	10.6 C	Bangor
514	120	110.5	2 E	264	32	7/19/2018	10.6 C	Bangor
473	120	109.3		243	29	7/20/2018	10.6 C	Bangor
420	120	107.3		395	23	7/13/2018	10.6 C	Bangor
420	120	102.9		395	23	7/14/2018	10.6 C	Bangor
420	120	97.5		395	23	7/14/2018	10.6 C	Bangor
420	120	97.1		395	23	7/13/2018	10.6 C	Bangor
420	120	92.3		395	23	7/13/2018	10.6 C	Bangor
461	80	87.9	1 F	237	29	7/19/2018	10.6 C	Bangor
522	80	87.5		99	12	7/23/2018	10.6 C	Bangor
420	80	84.1		386	23	7/14/2018	10.6 C	Bangor
478	80	81.3	1 S	91	11	7/23/2018	10.6 C	Bangor
467	80	76.6		240	29	7/20/2018	10.6 C	Bangor
544	80	75.6		103	13	7/23/2018	10.6 C	Bangor
420	80	74.5		386	23	7/14/2018	10.6 C	Bangor
447	80	73.5		229	28	7/19/2018	10.6 C	Bangor
420	80	72.3		386	23	7/13/2018	10.6 C	Bangor
407	80	71.5		209	26	7/20/2018	10.6 C	Bangor
420	80	70.7		386	23	7/14/2018	10.6 C	Bangor
408	80	70.6		209	25	7/19/2018	10.6 C	Bangor
410	80	67.7		210	25	7/20/2018	10.6 C	Bangor
420	80	66.0		386	23	7/13/2018	10.6 C	Bangor
420	80	62.8		386	23	7/13/2018	10.6 C	Bangor
495	40	41.4	1 T	94	11	7/23/2018	10.6 C	Bangor
473	40	41.0	2 E	243	29	7/19/2018	10.6 C	Bangor
420	40	40.7		385	23	7/14/2018	10.6 C	Bangor
557	40	39.4	1 S 1 T	106	13	7/23/2018	10.6 C	Bangor
432	40	38.5	2 F	222	27	7/19/2018	10.6 C	Bangor
420	40	38.0		385	23	7/14/2018	10.6 C	Bangor
435	40	37.9	1 E	223	27	7/20/2018	10.6 C	Bangor
490	40	37.9		93	11	7/23/2018	10.6 C	Bangor
438	40	37.5	3 E 1 T	225	27	7/19/2018	10.6 C	Bangor
420	40	36.6		384	23	7/14/2018	10.6 C	Bangor
507	40	34.6		260	32	7/20/2018	10.6 C	Bangor
420	40	34.3		385	23	7/13/2018	10.6 C	Bangor

480	40	34.1	1 T	246	30	7/20/2018	10.6 C	Bangor
420	40	34.0		385	23	7/13/2018	10.6 C	Bangor
420	40	31.9		384	23	7/13/2018	10.6 C	Bangor

Table 4.10. Probit and mortality proportion across methyl bromide exposures.

Probit and mortality proportion were calculated based on the methods of Couey and Chew for each measured methyl bromide exposure with $\pm$ 1.96s (95% confidence interval). Individual life stages were pooled together based on work indicating no most-tolerant life stage.

Table 4.10				
		-1.96s	$\bar{x}$	+1.96s
108.9 ± 7.0 2 survivors	Treated	3754	3928	4102
	Mortality	0.9983	0.9984	0.9985
	Probit	7.93	7.95	7.96
74.8 ± 7.3 2 survivors	Treated	3770	3944	4118
	Mortality	0.9983	0.9984	0.9985
	Probit	7.93	7.95	7.96
37.2 ± 2.8 13 survivors	Treated	3838	4018	4198
	Mortality	0.9946	0.9949	0.9951
	Probit	7.55	7.57	7.58

Table 4.11. Summary of treatment results as probabilities and probit analyses.

Percent and probit mortality of blueberry maggot (BBM) after each event was calculated at the 95% confidence level (95% CL). Observed survival is the simple proportion of BBM survivors in BBM treated. $P(E_x)$ is the probability of finding a surviving BBM after event X at the 95% CL.

Table 4.11				
Event X	Observed survival (%)	$P(E_x)$ (95% CL)	Percent mortality (95% CL)	Probit (95% CL)
Pesticide treatment	0.13	0.59	99.41	7.52
Postharvest methyl bromide	0.05	0.16	99.84	7.95

Table 4.12. Treatment results tabulated as joint probabilities associated with a respective series of events.

The joint percent and probit mortality of blueberry maggot was calculated from the independent probabilities of pesticide applications and subsequent methyl bromide fumigation at the 95% confidence level (CL). $P(E_1+E_2)$ is the probability calculated from the multiplication of the simple probability of each event.

Table 4.12			
Joint events	$P(E_1 + E_2)$	Percent mortality (%) (CL 95%)	Probit (CL 95%)
$\sum$ pesticide + methyl bromide	0.997207	99.9974	9.05

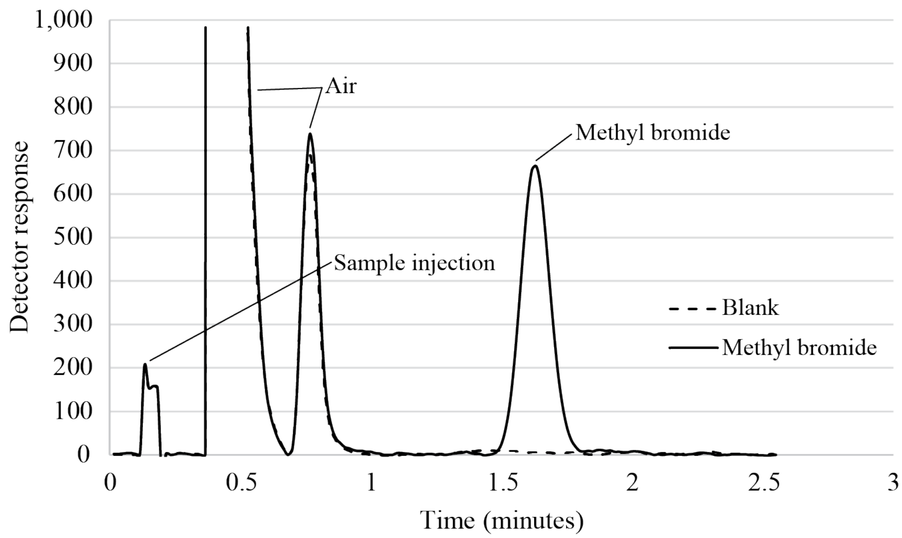


Figure 4.1. Chromatograms of a theoretical 7.34 mgL^{-1} methyl bromide standard and a blank

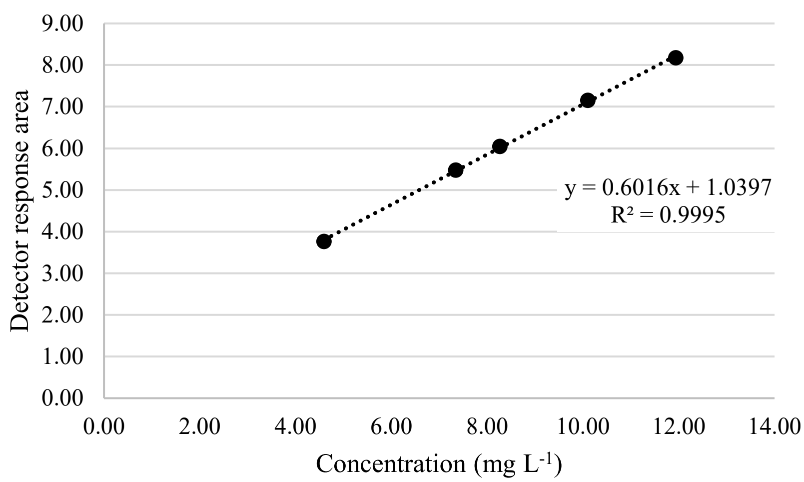


Figure 4.2. Sample methyl bromide calibration curve

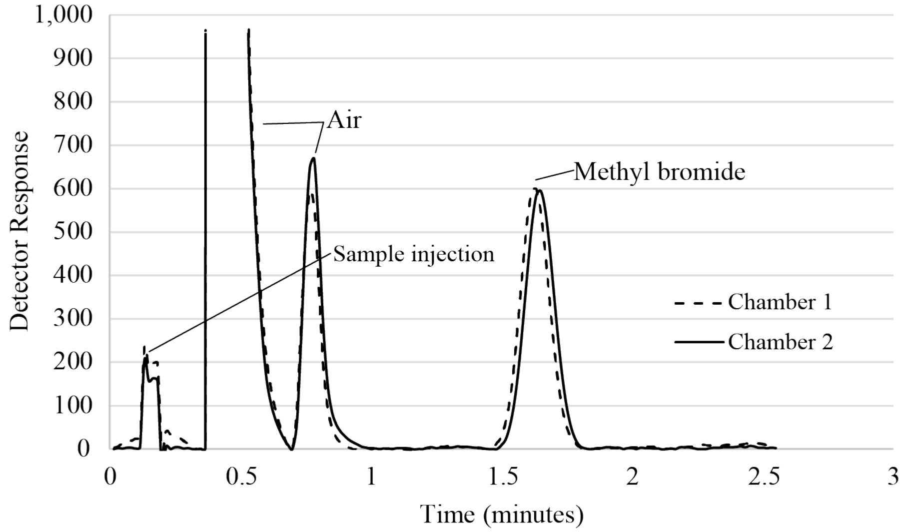


Figure 4.3. Chromatograms from two fumigation chambers dosed with a theoretical 7.34 mgL^{-1} methyl bromide

Part 2: Brown marmorated stink bug, *Halyomorpha halys* (Stål), case study

Chapter 5: Ethyl formate dilution in carbon dioxide for fumigation control of brown marmorated stink bug, *Halyomorpha halys* (Stål) (*Hemiptera: Pentatomidae*)

5.1. Abstract

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), has caused significant agricultural damage to numerous hosts, so producers seek to limit its spread. Where established, BMSB can also cause substantial urban and commercial disturbance, as overwintering adults may seek refuge inside dwellings, covered spaces, vehicles, and consignments. Phytosanitary authorities are most concerned with the importation of "hitchhiking" adults in this refugia, with certain countries requiring a quarantine treatment to mitigate risk. This study explores fumigation with ethyl formate (EF), applied as a 16.7% by mass dilution in carbon dioxide, for control of adult BMSB. The induction of diapause, to simulate overwintering physiology, resulted in a 2- and 3-fold increase in tolerance of adults toward the EF fumigation at 10±0.5 °C ($\bar{x} \pm 2s$) lasting for 8 and 12 h, respectively. However, a decreased tolerance (0.7- fold) of diapausing specimens was observed for a 4-h duration. Diapausing and non-diapausing adult BMSB can be controlled at the probit 9 level if the EF headspace concentration, [EF], is maintained ≥ 7.7 mgL^{-1} for 12 h at 10±0.5 °C ($\bar{x} \pm 2s$). If the duration is shortened to 4 h, [EF] must be maintained ≥ 14.7 mgL^{-1} over the course of fumigation. The toxicity of the EF fumigation can change for different physiological states of the same life stage. Respective to the physiological state of adults, this study identifies

how the applied dose and/or treatment duration can be modulated (i.e., tuned) to ensure adequate toxicological efficacy toward adult BMSB infesting hosts or refugia at temperatures > ca. 10 °C.

5.2. Introduction

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), (Hemiptera: Pentatomidae), is a generalist pest that has caused significant agricultural damage.[92] Moreover, BMSB has caused substantial urban and commercial disturbance, as overwintering adults may seek refuge inside of dwellings, covered spaces, vehicles, and consignments.[93–95] BMSB is regulated as a quarantine pest in certain countries that require phytosanitary treatments of potential hosts and refuges to mitigate risk. Australia (AUS) and New Zealand (NZ) are particularly concerned that treatments control "hitchhiking" BMSB in imported consignments and have identified those containing overwintering aggregations as the primary risk.[96] As of 2019, the only live BMSB intercepted in AUS are those departing from the Northern Hemisphere during winter months, likely due to the suppression of feeding that coincides with diapause. The use of heat treatments as well as postharvest fumigations with methyl bromide and sulfuryl fluoride are approved for control,[96,97] however heat treatments cannot alone meet the demand, and methyl bromide is incompatible with certain consignments that contain materials with reactive sulfur species such as luxury vehicles with leather seats or some types of vulcanized rubber.[98] Sulfuryl fluoride is registered in the USA for the disinfestation of vehicles and other consignments (ProFume®, Douglas Products, Liberty, MO) and a 12-h treatment has been used since ca. 2015 to control BMSB in AUS- and NZ-bound consignments, which have an annual value of 2 billion $US.[99] This sulfuryl fluoride use was begun in the European Union, particularly to satisfy the import requirements of AUS and NZ.

From the perspective of an on-arrival treatment in AUS and NZ, sulfuryl fluoride is not registered, but ethyl formate (EF), commercially formulated as a 16.7% by mass dilution in carbon dioxide and licensed as eFUME™ (Draslovka, North Melbourne, AUS), has the required registration and therefore could be used to control BMSB in the risk pathway described above.

The commercial use of EF as a postharvest fumigant, first published in 1925 by Neifert, Cook, Roark, and Tonkin to control grain weevils,[100] was overshadowed by the introduction of methyl bromide (MB) in the 1940s,[101] having just a few noteworthy uses including the disinfestation of dried fruit. The phase-out of MB, following the ratification of the Montreal Protocol in 1992, has renewed interest in EF with recent publications reporting its efficacy across a variety of commodities and pests.[10-24] Coetzee et. al. recently explored the use of EF formulated with nitrogen to treat containers at dockside as well as in-transit.[113] Ethyl formate is a colorless liquid with a pleasant, banana-like odor and a boiling point of 54.1 °C. Having a flash point of 2.3 V/V% in air,[114] the use of EF has long been accompanied by dilution in carbon dioxide for the purpose of fire suppression,[115] and to act as a propellant. Carbon dioxide affects insects in many ways as reviewed by Nicolas.[11] Carbon dioxide may influence fumigant toxicity, and such potential must be evaluated for each type of insect (life stage, age, etc.) as well as each co-fumigant.[116] The need to evaluate the impact, if any, of carbon dioxide carrier gas on the toxicity of EF toward insects is well documented.[32] Ethyl formate has been designated by the US Food Drug Administration as generally recognized as safe (GRAS).[117] This designation makes it an attractive option, relative to MB and sulfuryl fluoride (SF), at certain port facilities. Here we report the efficacy of EF toward BMSB adults at 10±0.5 °C ($\bar{x} \pm 2s$), the minimum treatment temperature requested by the registrant of eFUME™ and the NZ authorities, in an effort to guide the establishment of fumigation parameters that ensure BMSB-free goods are received, irrespective of the season during which the goods are shipped.

5.3. Materials and methods

5.3.1. Insects

Insects were from colonies located in the Contained Research Facility at University of California, Davis (Davis, CA), a Biosafety Level (BSL) III quarantine facility. The origin and rearing of these insects were detailed in Abrams et al.[93,118] A total of 5509 non-diapausing BMSB and 2814 diapausing BMSB were treated. There were 989 non-diapausing BMSB and 478 diapausing BMSB used as controls (Tables S5.1 and S5.2).

5.3.2. Induction and confirmation of diapause

Diapause was induced in BMSB adults and confirmed by dissecting non-treated female control specimens and examining their reproductive tracts for indication of development and mating as previously reported methods (Table S5.1).[93,118]

5.3.3. Chemicals and chemical analysis

A 250-mL bottle of EF was obtained from Sigma-Aldrich (97% purity, impurities unlisted, St. Louis, MO, USA), and a 136-kg cylinder of carbon dioxide gas was commercially sourced (Airgas, Fresno, CA, USA). Ethyl formate concentration in chamber headspace, [EF], was measured using a gas chromatograph coupled with a flame ionization detector (GC-FID). Detector response and retention time were determined before each experiment in calibration studies by diluting known volumes of liquid EF into volumetric gas vessels. The integral of peak area, referenced relative to linear least squares analysis of a 5-point concentration–detector response curve, was used to determine [EF].[85] Ethyl formate headspace concentration was recorded as the mean from duplicate samplings at each temporal interval for each trial (vide infra). Analyses were conducted with an HP 6890. The split-splitless injector in splitless mode was held at 160 °C. Samples were introduced using a gas sampling port (150 °C) with a 1-mL sample loop. A capillary RTX-VMS analytical column (L = 30 m, ID = 530.00 µm) (Restek, Bellefonte, PA, USA) was held at 200 °C for 2 min receiving 5 mL min^{-1} He flow, and the FID detector was held at 250 °C with 30 mL min^{-1} hydrogen, 400 mL min^{-1} air, and 30 mL min^{-1} nitrogen make-up.

The limit of blank (LOB) and limit of detection (LOD) for EF on GC-TCD were determined from instrument signal response according to the method of Armbruster and Pry.[80] The HP 6890, described above, was unavailable, and a Varian 3800 with FID was used as a proxy. Samples were introduced through a gas sampling port (150 °C) with a 1-mL sample loop. A packed OV-101 on Gas Chrom-Q 100-120 mesh analytical column (L = 1.8 m, ID = 2.1 mm) was held at 160 °C for 1 min receiving 35 ml min^{-1} He flow, and the FID detector was held at 165 °C. with 30 mL min^{-1} hydrogen, 300 mL min^{-1} air, and 30 mL min^{-1} air make-up. See section 4.3.4 for details on LOB and LOD calculations.

5.3.4. Exploratory fumigations

To generate exposure-mortality response data, a series of laboratory-scale exploratory fumigations were conducted in a matching set of six modified Labonco® 28.32-L vacuum chambers (Labconco Corporation, Kansas City, MO, USA) housed in a walk-in environmental room (BIOCOLD Environmental, Fenton, MO) with programmable temperature.[81] Temperature was set to 10.0±0.3°C. Fumigations were conducted at normal atmospheric pressure for 4, 8, and 12 h, each over a range of applied doses ≤ 12.6 mgL^{-1}. Adults were collected from rearing enclosures, and groups of 10 to 15 specimens were each transferred to 950-mL plastic cup cages modified with 25-mm diameter cloth mesh gas-portals on the lid and sides. Shelled walnuts, almonds, and a small cup of water with a cotton wick were added to each cage for nutrients. Cages were labeled respective to the treatment parameters (chamber #, dose, duration, etc.). Chambers loaded with caged specimens, cages containing specimens that were not subject to treatment (i.e., non-treated controls), source-gas cylinders, and gas-tight syringes (Hamilton® 500-, 1000-, or 1500-mL, Reno, NV, USA) were acclimated to fumigation temperature, or tempered, for 12 h prior to treatment. Chamber air temperature was confirmed prior to fumigation by a HOBO data logger (HOBOware version 2.7, Bourne, MA, USA). Accuracy of HOBO data loggers was checked with the ice-water immersion method as described in American Society for Testing and Materials (ASTM) E563-11, the day before the test. Chamber lids were then secured in preparation for treatment.

A vacuum of ca. 10 to 13 kPa was established in each chamber. Syringes (Hamilton® 100- or 500-μL) were filled with a volume of liquid EF to achieve the requisite applied dose, C_0, in chamber headspace, as predetermined in calibration studies. The syringe was inserted through a rubber septum covering the gas portal atop the chamber, and liquid EF was injected onto a glass watch dish placed on top of the cage directly below the injection port. A gas-tight super-syringe (Hamilton® 500-, 1000-, or 1500-mL) was filled with a predetermined volume of gaseous carbon dioxide and then fitted to a LuerLok® sampling valve, which was subsequently opened so that the carbon dioxide was steadily drawn into the chamber. The syringe was then removed without closing the valve, and normal atmospheric pressure was re-established in the chamber before the valve was closed; this marked the beginning of the exposure period.

Samples (10 mL) of chamber headspace were taken through a LuerLok® valve using a B-D® 30-mL graduated gas-tight syringe (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and quantitatively analyzed with GC-FID as described above. An initial sampling of [EF] was analyzed within 10 min of fumigation commencement, to afford $[EF]_0$, and thereafter, sampling occurred at standard 2- or 4-h temporal intervals of time, *t*, depending on the experiment duration (e.g., $[EF]_0 \approx [EF]_{t \leq 10\ \mathrm{min}}$, $[EF]_{t = 2\ \mathrm{h}}$, $[EF]_{t = 4\ \mathrm{h}}$, etc.). Fumigant exposures were expressed as concentration (*C*)×time (*t*) products (mgL^{-1} h). "Measured" exposure (Ct_m) was calculated by the method of Monro[98] based on measurement at the temporal intervals, $[EF]_t$, whereas "theoretical" exposure (Ct_{th}) was calculated as the product of the applied dose (C_0) and the fumigation duration (*t*).

Following the final sampling of $[EF]_t$, chamber valves were opened to atmosphere, a 1-h aeration period was initiated, and chamber lids were then opened. After aeration, the cages containing treated and non-treated control specimens were retrieved and returned to the greenhouse separate from colonies until mortality was assessed. Specimens were frozen and discarded after assessment.

5.3.5. Ethyl formate sorption kinetics

The EF sorption kinetics in the "naked" chamber fumigations were observed to follow first-order kinetics. The natural log of [EF] data collected from fumigations of diapausing and non-diapausing BMSB was plotted against time to find the observed sorption rate constant, k_{OBS}, as the slope of the linear least-squares regression ($\ln([EF]_t) - \ln\left([EF]_{t_0}\right) = k_{OBS}t$).

5.3.6. Mortality

Mortality of non-fumigated and fumigated specimens was diagnosed by lack of prodding induced motion assessed at 3- and 5-d intervals following fumigation. The mortality response (*r*) was calculated by subtracting the number of survivors (*s*) from the number of fumigated specimens (*n*). Control mortality was treated numerically using Abbott's method,[119] as described by Finney.[82]

5.4. Results and discussion

5.4.1. GC-FID analysis

Detector signal from blank samples ($n = 15$) from 0.57 to 0.79 min, corresponding with the retention time of EF ($t_r = 0.614$ min), were used to calculate $\bar{x}_{blank} \pm s_{blank}$ as 455 ± 174 μV, and the LOB was 743 μV. The LOD was 1392 μV, and triplicate standards of 0.0286 mgL^{-1} EF, the lowest [EF] quantified in fumigation studies, produced a mean peak detector signal of $12{,}634 \pm 721$ mV ($\bar{x} \pm s$), 9-fold greater than the LOD (Figure 4.1).[80]

A matched set of five modified 28.32-L fumigation chambers with circulation fans were dosed to yield [EF] of 0.027 mgL^{-1} after 5 minutes. The integral of peak area, referenced relative to linear least squares analysis of a 5-point concentration–detector response curve (Figure 5.2), was used to determine the mean [EF] concentration in the chambers as 0.0269 ± 0.0007 mgL^{-1} ($\bar{x} \pm s$) that yielded 99.6% of the target concentration, 0.027 mgL^{-1} (Figure 5.3).

5.4.2. Ethyl formate stability and sorption kinetics

The mean air temperature in the chambers was calculated across all trials; deviation in temperature was assumed to follow a normal distribution with the estimated margin of error reported as 10 ± 0.5 °C ($\bar{x} \pm 2s$).[120] Consistent with its well-documented potential for sorption,[32,84,107,112,113,121–125] [EF] did not remain constant over the duration of fumigation, therefore, $(Ct)_m$ (Tables S5.2 & S5.3) was used in the exposure-mortality regression modeling to calculate $(Ct)_p$ (Table 5.1). For these "naked" fumigations (i.e., containing no commodity), $(Ct)_m$ was $31 \pm 9\%$ ($\bar{x} \pm s$) lower than the theoretical exposure ($(Ct)_{th}$).

Half-lives, based on first-order kinetic rate constants, k_{OBS}, for 4-, 8-, and 12-hour fumigations were 9.3, 14.2, and 15.9 h, respectively (Figure 5.4). The loss of EF over the course of these fumigations could have resulted from hydrolysis, condensation, sorption (e.g., into insects, water, or food), or combinations thereof. The rate of sorption in commercial fumigation scenarios depend on the material composition of fumigated consignments. Additionally, %RH may play a role in the rate of EF sorption, especially at port facilities or in-transit sea-container fumigations. Any condensation will provide a medium for sorption as well as hydrolysis (Figure 5.5). Ethyl formate that is hydrolyzed into formic acid and ethanol will not desorb post-fumigation, as esterification is improbable. The half-life of EF hydrolysis in water is 24 h at 30 °C near pH 7.[126]

Methods used to predict sorption of MB into fresh blueberries and packaging in Chapter 4 will not be applicable to consignment fumigations, as refugial consignments constitute a variety of materials and will not have a standardized load composition. Fumigators will need to rely steady-state applications or [EF] monitoring with re-dosing to maintain the requisite headspace concentrations.

5.4.3. Exposure-regression modeling

Across this data, mean mortality of non-fumigated controls was $16.3 \pm 36.8\%$ $(\bar{x} \pm 2s)$. Exposure-regression modeling was conducted according to the methods of Finney in Probit Analysis[82] using a program developed in-house with Mathematica (Wolfram Research, Champaign, Illinois).[127] Note that the "improved" Mathematica-based statistical treatment is novel and distinct from reported preliminary research, which was analyzed with Polo Plus (LeOra Software, Berkeley, California) and included fewer experimental replications.[128] Improvements included longer floating-point values to reduce rounding errors, > 200 groups, user-selectable mortality predictions, and modified Haber's rule parameter calculations. Table 1 lists the projected exposures ($(Ct)_p$) to cause 95, 99, and 99.9968% mortality in the treated population (respectively LE_{95}, LE_{99}, to LE_{P9}) and the corresponding estimates of the bounds (upper (UL) and lower (LL) limits) at the 95% CL. Probit 9 (P9) projections, where a = 99.9968% mortality,[79,83] are often prone to bounds that span an order of magnitude, or greater, which can decrease confidence in the utility of probit analysis. Moreover, it is difficult to confirm probit 9 projections when test insects are difficult to rear in numbers approaching 100,000, such as the case for BMSB (diapausing and non-diapausing). In this case, the heterogeneity (H) and χ^2 indicate that the probit model fitted the observed data, with UL's exceedance never more than 2-fold.

5.4.4. Exploratory fumigations: Non-diapausing brown marmorated stink bug

Fumigations lasting 4, 8, or 12 h were conducted to target non-diapausing adults (Table S5.2). Lethal exposure ratios (LERs) were calculated using $(Ct)_p$ with (±) 95% confidence intervals (CI) and used to identify how the mortality response of non-diapausing BMSB adults toward EF fumigation potentially changes as a function of C and t. LERs calculated for 4-h durations relative to 8- and 12-h durations had ratios of 1.20 and 1.28 respectively at a = P9% (Figure 5.6). Haber's rule forms the basis for relating C, t, and ultimately Ct to lethality (ω), at least with respect to fumigation science.[129–131] Its most familiar expression, $Ct=\omega$, takes a form where ω (mgL^{-1} h) is an empirical level of response for a given endpoint, in this case, a proxy for the mortality specific to a Ct. The modified Haber's rule takes the form, $C^z t=\omega$, where z (unitless) modifies the relative impact of C for a specific toxicant-organism pairing. Experimental data support the modified Haber's rule described above, for each $(Ct)_p$ over the range of a, 50 to P9% as the negative slope ($-\Delta\, m/x = z_p^a$) from a least-squares analysis of "log C_p" plotted versus "log t" were linear and had correlation coefficients (r^2) > 0.94, where $C_{p'} = (Ct)_p\, t^{-1}$ (Table 5.2).

Values of z_p^a, respective to a particular projection (z_p^{50}, z_p^{60}, z_p^{70}, etc.) decreased over the range 1.00 to 0.81 as a increased for non–diapausing adults over the range 50 to P9% (Table 5.2).

When t contributes more than C toward efficacy, $z_p^a < 1$ (unity), a result supported by the LERs where an increase in t results in a lower required Ct to achieve the same lethality (Figure 5.6). As a increased, values of $\omega_{p,t}^a$ also increased. Small variation in $\omega_{p,t}^a$ for a particular a were likely the result from variability inherent to regression modeling, as discussed by Bliss.[129] Table 5.2 lists duration-specific $\omega_{p,t}^a$ values for each a (i.e., $\omega_{p,4}^{50}$, $\omega_{p,8}^{50}$, $\omega_{p,12}^{50}$, etc.) as well as the mean values across a (i.e., $\overline{\omega}_p^{50}$, $\overline{\omega}_p^{60}$, $\overline{\omega}_p^{70}$, etc.). A single factor-analysis of variance (ANOVA) at the 95% CL comparing the mean values was significant, ($F_{(14, 30)} = 5788$; $P = 2\times10^{-16}$), suggesting the overall mean ω_p value ($\omega_p = 18.4\pm8.0$ $(\text{mgL}^{-1})^z$ h; $\bar{x} \pm 2s$) cannot be used to estimate a particular lethality. A post hoc Tukey's multiple means comparison (α = 0.05) indicated significant differences between all groups of $\omega_{p,t}^a$ for a = 10 to P9% (Table 5.2).

The modified Haber's rule was rearranged to calculate the minimum headspace concentration (C') required to achieve a desired percentage mortality (a) for a chosen fumigation duration (t):

$$C' = \left(\frac{\overline{\omega}_p^a}{t}\right)^{\frac{1}{z_p^a}}$$

(eq. 5.1)

The parameters cited above (a = P9%, $\overline{\omega}_p^{P9}$= 35.6 $(\text{mgL}^{-1})^z$ h, z_p^{P9} = 0.81) were selected to yield conservative estimates of required applied dose and/or the treatment duration to yield mortality of 99.9968% (i.e., a = P9) mortality (Figure 5.7). Fumigation results for the non-diapausing specimens indicate a treatment at air temperature ≥ 10.0 ± 0.5 °C ($\bar{x} \pm 2s$) for a 12-h duration will yield probit 9 control (a = P9) if [EF] is maintained ≥ 3.82 mgL^{-1} (lower limit (LL): 2.93 mgL^{-1};

upper limit (UL): 5.99 mgL^{-1} at the 95% CL). If the duration is shortened to 4 h, [EF] ≥ 14.7 mgL^{-1} is required (LL: 12.0 mgL^{-1}; UL: 19.8 mgL^{-1} at the 95% CL) (Figure 5.7).

5.4.5. Exploratory fumigations: Diapausing brown marmorated stink bug

Diapausing specimens, which were not considered above, may be of regulatory concern to countries importing goods that depart during winter months as rationalized above. Fumigations lasting 4, 8, or 12 h were conducted to concurrently target diapausing adults, as well as non-diapausing adult fumigated controls (Table S5.3). Across this data, mean mortality of non-treated controls was $4.6 \pm 17.3\%$ and $20.3 \pm 27.8\%$ $(\bar{x} \pm 2s)$, respectively, for diapausing and non-diapausing specimens. Exposure-mortality regression modeling was conducted as above (Table 5.1). Table 5.1 lists the $(Ct)_p$ to cause 95, 99, and 99.9968% mortality in the treated population (respectively LE_{95}, LE_{99}, to LE_{P9}) and the corresponding estimates of the bounds (UL and LL) at the 95% LOC. Probit models fitted the observed data with UL's exceedance never more than 2-fold.

LERs (±95% CI) using $(Ct)_p$ were calculated to identify how the mortality response of diapausing BMSB adults toward EF fumigation potentially changed as a function of *C* and *t*. LERs calculated for 4-h durations relative to 8- and 12-h durations had ratios of ca. 0.61 and 0.42 respectively at *a* = P9% (Figure 5.6). Moreover, LERs (±95% CI) were used to compare the relative efficacy of diapausing versus non-diapausing specimens ($(Ct)_{p,D}/(Ct)_{p,ND}$) across the three treatment durations. Diapause resulted in a ca. 2.2- and 2.8-fold increased tolerance for 8- and 12-h fumigations respectively at *a* = P9%, but the LER was 0.7 for a 4-h fumigation (Figure 5.8).

Values of z_p^a, respective to particular projections (z_p^{50}, z_p^{60}, z_p^{70}, etc.) increased over the range, 3.27 to 4.27, as *a* increased for diapausing adults from 50 to P9% (Table 5.2). A z_p^a > 1 indicates that C has a greater contribution toward treatment efficacy, relative to t, which is supported by the LERs where increasing t results in an increased Ct to achieve the target lethality (Figure 5.6). It is interesting to note that z varied markedly depending on the physiological state of BMSB (Table 5.2). Large differences in values of $\omega_{p,t}^{a}$ between levels of a = 50 to P9 are a result of the large z_p^a since each $\omega_{p,t}^{a}$ is calculated as $\omega_{p,t}^{a} = C_{p'}^{z_p^a} t$ ($C_{p'}$ defined above) and $C_{p'}$ is raised by the exponent z_p^a. To address the > 100-fold span observed for $\overline{\omega}_p^a$, values were log transformed prior to a single-factor ANOVA, which indicated significant difference between means of $\overline{\omega}_p^a$ ($F_{(8,\ 18)}$ = 835.7 ; P = 2.0×10^{-16}), suggesting that the overall mean ($\overline{\omega}_p$ = $9.6\times10^{3}\pm4.4\times10^{4}$ $(\mathrm{mgL^{-1}})^{z}$ h ($\bar{x} \pm 2s$)) cannot be used to estimate probit 9 mortality. A post hoc Tukey's multiple means comparison (α = 0.05) was used to identify groups of $\overline{\omega}_p^a$ with a statistical difference. Only two pairs a = 50 vs. 60 and a = 80 vs. 85 were not statistically distinct, but all others were significantly distinct (Table 5.2). These results are consistent with those above, suggesting subsequent predictive calculations should be specific to a desired value of a.

Modified Haber's rule parameters were chosen based on the same criteria as non-diapausing BMSB (vide supra). Fumigation results for the diapausing specimens indicate an EF treatment with air temperature ≥ 10.0 ± 0.5 °C ($\bar{x} \pm 2s$) for a 12-h duration will yield probit 9 control of adult BMSB (a = P9, z_p^{P9} = 4.27 and $\overline{\omega}_p^{P9}$ = $7.2\times10^{4}\pm1.8\times10^{4}$ $(\mathrm{mgL^{-1}})^{z}$ h ($\bar{x} \pm 2s$)) if [EF] is maintained ≥ 7.7 $\mathrm{mgL^{-1}}$ (LL: 6.48 $\mathrm{mgL^{-1}}$; UL: 11.03 $\mathrm{mgL^{-1}}$ at the 95% CL). If the duration is shortened to 4 h, [EF] must be maintained ≥ 9.9 $\mathrm{mgL^{-1}}$ (LL: 8.98 $\mathrm{mgL^{-1}}$; UL: 11.48 $\mathrm{mgL^{-1}}$ at the 95% CL). Plots of the experimentally determined relationship between dose ($\mathrm{mgL^{-1}}$) and duration (h) for diapausing and non-diapausing BMSB to achieve probit 9 mortality shows that the two curves intersect at 5.9 h with a maintained [EF] of 9.1 $\mathrm{mgL^{-1}}$ (Figure 5.7). This has important implications for fumigant applicators as the exposure required is not dependent on a single most EF tolerant physiological state. Instead, fumigators will need to choose an exposure curve for diapausing or non-diapausing BMSB based on the duration of fumigation.

5.4.6. Comparison of Haber's rule parameters

Insecticidal fumigants have different mechanisms and modes of action, which are known to influence the magnitude of z, a proxy for the relative importance of C versus t. Efficacy data contributed by many investigators across a variety of targeted insect pests indicates that $z \cong 1$ for MB (at least when $t < 6$ h) and is consistent with a mechanism involving alkylation of nitrogen- and sulfur- containing biomolecules.[132] For phosphine, z changes as a function of C.[133,134] Winks operationally defined the "narcosis threshold" as the region where $z \cong 0$, whereby increases or decreases in phosphine concentration did not change the duration required for the particular level of control (e.g., 99% mortality).[133,134] It is important to note, however, that phosphine is often operationally applied at a concentration below the "narcosis threshold", where $z < 1$ and mitochondrial inhibition is the predominate activity.[133,134] Similarly, understanding the magnitude of z for SF and EF must be linked to toxic action. In previous research,[93] we explored the modified Haber's Law parameters for disinfestation of adult BMSB with SF at temperatures > ca. 10 °C. Interestingly, we found that from LE_{50} to LE_{P9} z ranged from 0.73 to 0.59 for non-diapausing BMSB, and z decreased to 0.58 to 0.43 for specimens in diapause, indicating that diapausing BMSB, relative to non-diapausing BMSB, are more tolerant at short fumigation durations but less tolerant at long fumigation durations. Sulfuryl fluoride is hydrolyzed to yield fluoride ions, which inhibit glycolysis.[135–137] Without a mechanism to break down glucose, cells are forced to convert amino acids to members of the citric acid cycle to provide energy.[137] While unsustainable for long durations, the utilization of amino acids for energy might sustain diapausing BMSB for SF fumigations < 20 h and explain the smaller z value for diapausing BMSB relative to non-diapausing BMSB.[93] These results contrast the EF with carbon dioxide findings, where from LE_{50} to LE_{P9} z ranged from 1 to 0.8 for non-diapausing BMSB and from 3.3 to 4.3 for diapausing BMSB (Table 5.2). Ethyl formate is hydrolyzed into formic acid and ethanol, and the active compound, formic acid, inhibits cytochrome C oxidase by the same mechanism as hydrogen cyanide.[102,109,138] While plant cells contain alternative oxidase, the enzyme is rare in insects, and without an alternative end to the electron transport chain, cell death would be more rapid than glycolysis inhibition.[139,140] Increased tolerance observed for non-diapausing BMSB could be due to higher relative metabolism more rapidly eliminating formic acid, however the protection offered by increased metabolism only improves survivorship for fumigation durations < 6 h as observed in Figure 5.7. At longer durations, increased metabolism likely increases the rate of damage to cells. However, much less literature is available on these fumigants than for MB or phosphine, which confounds generalization across insect species, life stages, and/or physiological state. As such, mechanisms specific to BMSB proposed above are speculative and based on general insect biology and research conducted on SF and EF in other arthropods. Molecular biology could expand our understanding and improve interpretations; however, such investigations are beyond the scope of these works, which were focused on providing regulators and fumigation service providers with efficacious treatments.

5.5. Conclusion

Probit 9 mortality projections are given as a relative metric, without intent to define the validity or potential success of a treatment. Ultimately, the importing country determines the level of control that must be demonstrated to mitigate risk associated with invasive insect pests per the

International Standards for Phytosanitary Measures 28 (ISPM 28).[43] In fact, several recent works discuss the "probit 9 paradigm" with respect to BMSB control.[141–143] While treatment criterion to ensure a probit 9 mortality are cited above, the predictive descriptions identify the parameters required to achieve other target levels of efficacy. For example, to achieve a level of control consistent with probit 8.7 (99.99% mortality) an [EF] ≥ 13.1 mgL^{-1} would have to be maintained for 4 h ($z_p^{P8.7}$ = 0.82 and $\overline{\omega}_p^{P8.7}$ = 33.21) or an [EF] of 7.18 mgL^{-1} for 12 h ($z_p^{P8.7}$ = 4.18 and $\overline{\omega}_p^{P8.7}$ = 4.6×10^4) to ensure that goods are received BMSB-free at this level.

With respect to commercial implementation of eFUME™ or other EF fumigation mixtures for BMSB control, authorities will work with industry to identify the parameters required for a technically efficacious treatment, which best conforms with logistical, operational, and regulatory constraints. Another consideration is to altogether ignore, or appreciate, the relative likelihood of encountering diapausing adults at the time the treatment. Relatively short fumigation durations offer the advantage of increased throughput, which is particularly at important at certain port facilities. If a treatment duration < 6 h is coveted, note that the compensatory increase in the amount of eFUME™ required for control of all BMSB adults is predominately driven by the requirement for individuals not in diapause. Results indicate such an approach would not reflect the most judicious use of eFUME™ when fumigating a consignment in the Northern Hemisphere during February, prior to oceanic export to AUS and NZ, for example. On the other hand, if the proposed fumigation was to occur upon arrival in AUS and NZ, greater biosecurity would be achieved by selecting the parameters that control even those individuals that may have broken diapause during the voyage. If fumigation durations > 6 h are acceptable, the design of operational parameters based on distinction of physiological state becomes even more difficult to rationalize, as the required applied dose (i.e., EF and carbon dioxide) and/or [EF] levels, as well as the operational relevancy of fumigant conservation, incrementally diminish with the lengthening of treatment time.

Whatever fumigation parameters are ultimately implemented, this work indicates the fumigation service provider should monitor [EF] to ensure a minimum level is maintained over the duration of the treatment. This may require repeated applications of EF or continuous, steady-state dosing. Although additional phytosanitary treatment demonstrations may be requested per guidelines of ISPM No. 28,[43] interest from AUS and NZ importers and authorities will ultimately drive the adoption of EF mixtures to disinfest consignments of BMSB. Upon commercial adoption, undoubtedly efforts will be made to ensure EF use for adult BMSB control is strategically optimized from the perspectives of food security, environmental health, and human health.

Table 5.1. Probit projected *Ct* exposures, $(Ct)_p$, for non-diapausing and diapausing *H. halys* adults.

Probit analysis of measured exposure $(Ct)_m$ versus mortality response was conducted for non-diapausing and diapausing *H. halys* adults following exploratory fumigations with eFUME™ (16.7% by mass ethyl formate dilution in carbon dioxide) for 4, 8, or 12 h at ca. 10.0 ºC. The tables list the projected exposures $(Ct)_p$ to cause 95, 99, and 99.9968% (P9) mortality in the treated population (respectively LE_{95}, LE_{99}, to LE_{P9}), the corresponding estimates of the bounds (upper (UL) and lower (LL) limits) at the 95% confidence level (CL), heterogeneity (*H*), and the Chi-squared fitting parameter (χ^2).

Non-diapausing

	4-h duration			8-h duration			12-h duration		
	$((mgL^{-1})^z h)$	95% CL		$((mgL^{-1})^z h)$	95% CL		$((mgL^{-1})^z h)$	95% CL	
	$(Ct)_p$	LL	UL	$(Ct)_p$	LL	UL	$(Ct)_p$	LL	UL
LE_{95}	21.7	20.0	24.1	20.4	18.2	24.1	19.6	17.4	23.5
LE_{99}	25.9	29.0	33.9	26.4	22.6	32.9	25.1	21.4	32.5
LE_{P9}	59.3	48.0	79.3	49.4	38.5	71.0	46.2	35.1	71.9
Treated	2453			1391			1665		
Control	414			269			306		
χ^2	588			312			331		
H	2.44			1.89			2.00		

Diapausing

	4-h duration			8-h duration			12-h duration		
	$((mgL^{-1})^z h)$	95% CL		$((mgL^{-1})^z h)$	95% CL		$((mgL^{-1})^z h)$	95% CL	
	$(Ct)_p$	LL	UL	$(Ct)_p$	LL	UL	$(Ct)_p$	LL	UL
LE_{95}	23.9	22.9	25.3	38.2	34.4	46.5	53.9	49.2	62.3
LE_{99}	27.7	26.1	30.0	44.6	38.8	58.7	63.5	56.2	77.3
LE_{P9}	39.9	35.9	45.9	65.2	51.7	104.7	94.7	77.7	132.4
Treated	1819			450			545		
Control	302			93			83		
χ^2	363			85			103		
H	2.04			1.92			1.98		

Table 5.2. Haber's parametrics for non-diapausing and diapausing *H. halys* adults following ethyl formate fumigation ca. 10.0 °C.

Haber's rule parameters associated with the eFUME™ fumigation (16.7% by mass dilution of ethyl formate in carbon dioxide) of non–diapausing and diapausing *H. halys* adults for different durations are listed below, including: z_p^a given as the negative slope (-Δ m/x) from a least-squares analysis of "log C_m" plotted versus "log t", linear correlation coefficients (r^2), and $\omega_{p,t}^a$ ((mgL^{-1})^z h) given as an empirical response to the probit-projected exposure $(Ct)_p$ resulting in a particular mortality percentage, a.

Non-diapausing BMSB

Mortality (%) (a)	z_p^a	r^2	ω_p^a ((mgL^{-1})^z h) 4 h	8 h	12 h	$\overline{\omega}_p^a$		s
50	1.00	0.9994	10.8	11.1	10.8	[a]10.89	±	0.15
60	0.99	0.9996	11.9	12.1	11.8	[b]11.93	±	0.14
70	0.97	0.9997	13.1	13.3	13.0	[c]13.11	±	0.13
80	0.96	0.9998	14.6	14.7	14.5	[d]14.60	±	0.11
85	0.95	0.9999	15.5	15.7	15.5	[e]15.57	±	0.09
90	0.93	0.9999	16.8	16.9	16.8	[f]16.84	±	0.07
95	0.92	1.0000	18.8	18.9	18.8	[g]18.85	±	0.04
99	0.88	1.0000	23.0	23.0	23.1	[h]23.01	±	0.06
P9	0.81	0.9996	35.7	35.1	35.9	[i]35.55	±	0.42

Diapausing BMSB

Mortality (%) (a)	z_p^a	r^2	ω_p^a ((mgL^{-1})^z h) 4 h	8 h	12 h	$\overline{\omega}_p^a$		s
50	3.27	0.98	431	388	450	[a]423	±	32
60	3.32	0.98	556	500	582	[b]546	±	42
70	3.37	0.98	737	660	774	[c]724	±	58
80	3.44	0.98	1037	924	1092	[d]1018	±	86
85	3.48	0.97	1288	1144	1359	[d]1264	±	110
90	3.54	0.97	1703	1508	1802	[e]1671	±	150
95	3.62	0.97	2619	2306	2784	[f]2569	±	243
99	3.79	0.96	6197	5402	6653	[g]6084	±	633
P9	4.27	0.94	7.3×10^4	6.2×10^4	8.1×10^4	[h]7.2×10^4	±	9.5×10^3

Superscript letters for $\overline{\omega}_p^a$ indicate groupings that are not significantly different according to a Tukey's multiple means comparison test (α = 0.05).

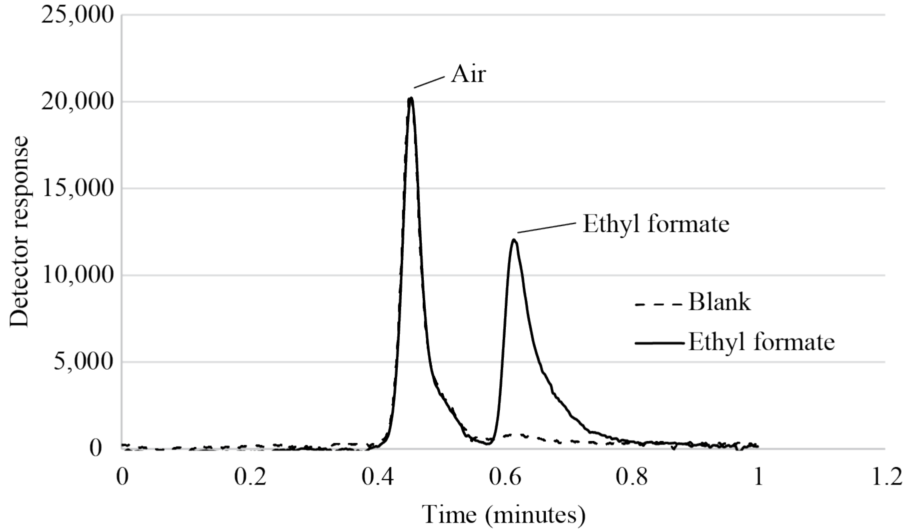


Figure 5.1. Chromatograms of a blank and a theoretical 0.0286 mgL^{-1} ethyl formate standard

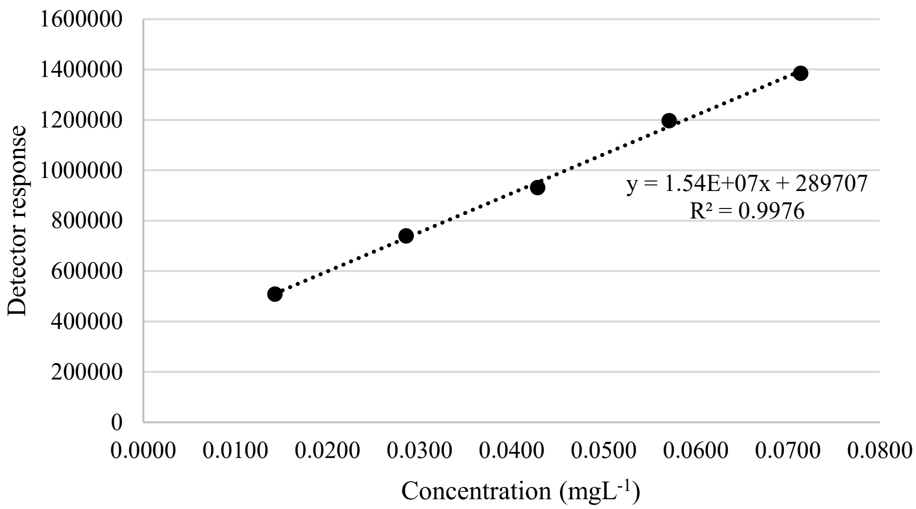


Figure 5.2. Sample ethyl formate calibration curve

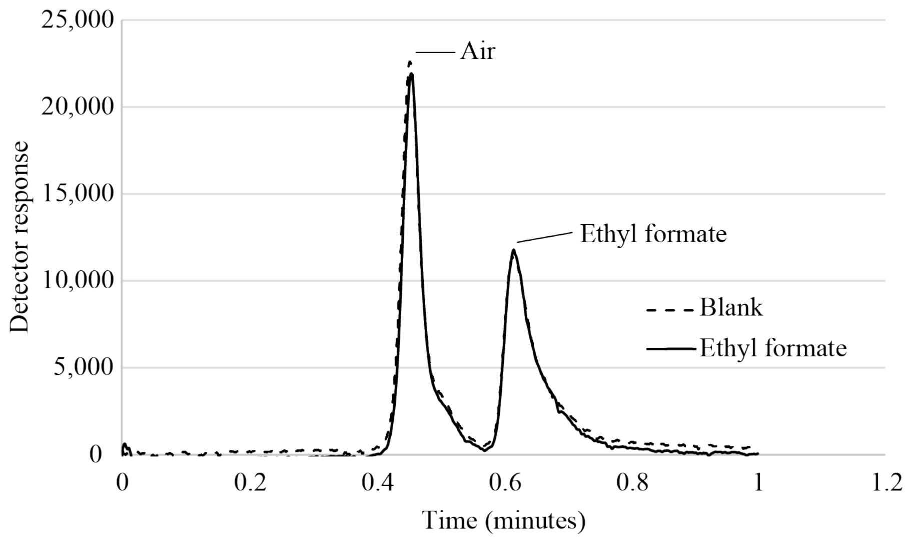


Figure 5.3. Chromatograms from two fumigation chambers dosed with a theoretical 0.027 mgL^{-1} ethyl formate

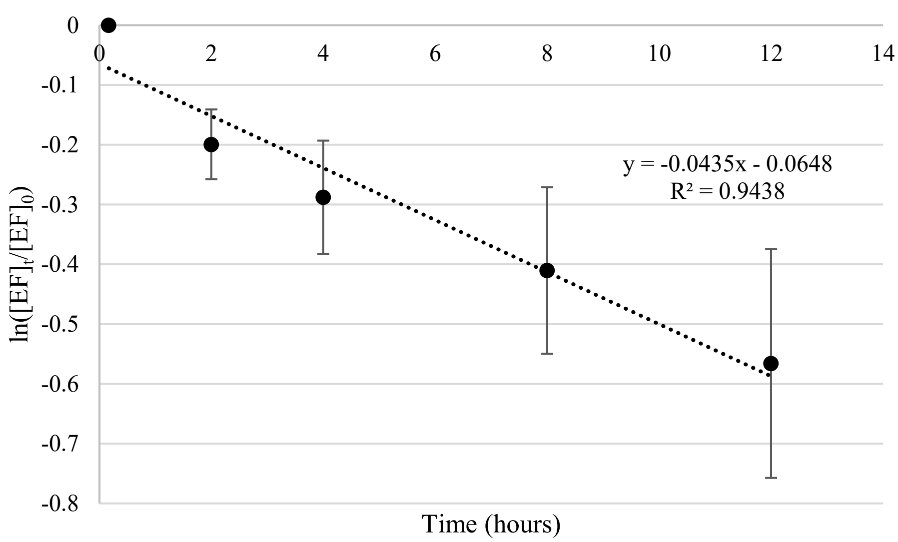


Figure 5.4. Linear first-order kinetic plot of ethyl formate headspace concentration

Concentration data across all fumigations, 4, 8, and 12 h, was plotted versus time as $\ln([EF]_t) - \ln\left([EF]_{t_0}\right)$. Ethyl formate sorption in "naked" chamber fumigations follows a first order kinetic plot after a high initial sorption rate.

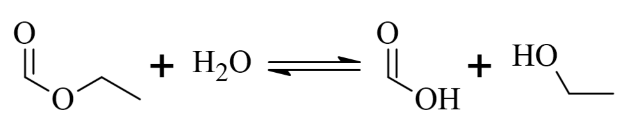


Figure 5.5. Hydrolysis of ethyl formate

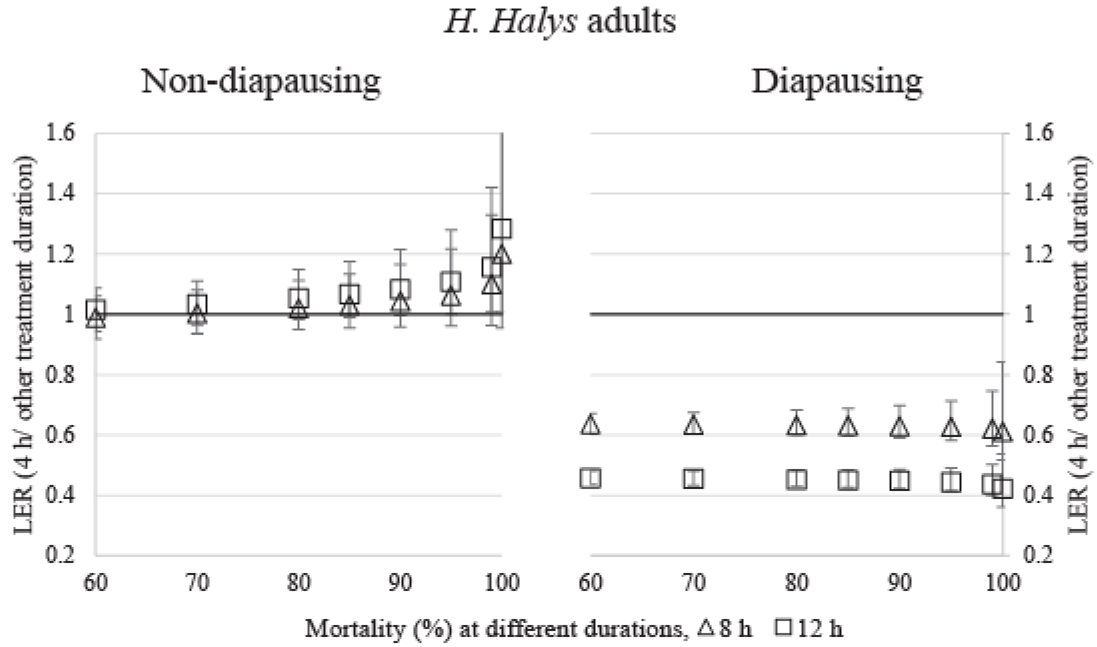


Figure 5.6. Relative susceptibility *H. halys* adults to ethyl formate fumigations of different durations.

Lethal exposure ratios (LERs) were calculated using probit projected exposures, $(Ct)_{p}$, ±95% confidence intervals to cause from 60 to 99.9968%(P9) mortality for non-diapausing and diapausing adult *H. halys* after exposure to eFUME^{TM} fumigation (16.7% by mass ethyl formate diluted in carbon dioxide). LERs for non-diapausing adult *H. halys* were > 1 indicating that time had a greater influence than concentration toward efficacy. Conversely, LERs for diapausing adult *H. halys* were < 1 indicating that time was less influential than concentration.

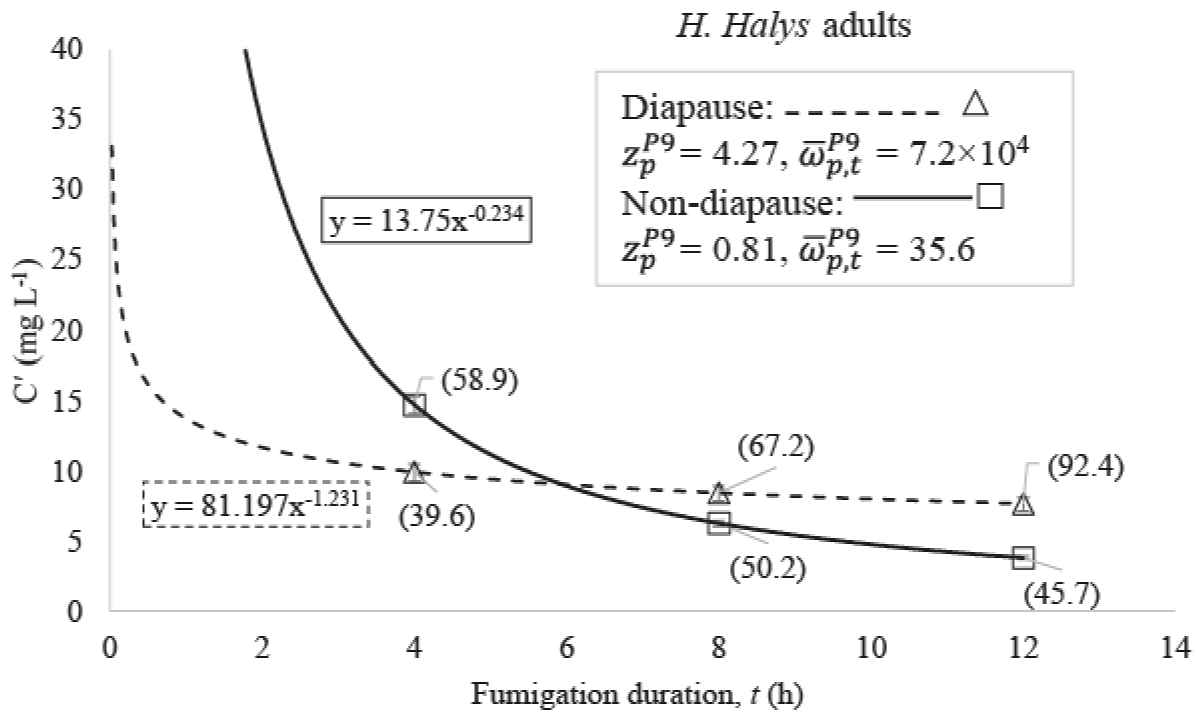


Figure 5.7. Relationship between minimum predicted ethyl formate headspace concentration (C') and treatment duration (*t*) at 99.9968% (P9) mortality for eFUME™ of *H. halys* adults.

The modified Haber's rule (rearranged to solve for C: $C = (\omega t^{-1})^{\frac{1}{z}}$), where $\omega = \bar{\omega}_p^{P9}$ and $z = z_p^{P9}$, was used to predict the minimum ethyl formate concentration in chamber headspace, C', which must be maintained over a given duration, *t*, to ensure a mortality level (*a*) of 99.9968% (P9) following fumigation of diapausing vs. non-diapausing *H. halys* adults with eFUME™ (16.7% by mass ethyl formate dilution in carbon dioxide) at air temperature of ca. 10.0 °C. Listed in parentheses, the corresponding calculated exposures, $(Ct)''$ ((mgL^{-1})^z h) for each treatment duration were calculated using Haber's rule parameters listed in the legend. Power least-squares analysis of diapausing and non-diapausing *H. halys* adults shows how the most ethyl formate-tolerant adult state changes as a function of fumigation duration, with an intersection at 5.9 h. Vertical error bars for both diapausing and non-diapausing *H. halys* adults are calculated as $\bar{\omega}_p^{P9} \pm 2s$. Error bars smaller than symbols may not be visible.

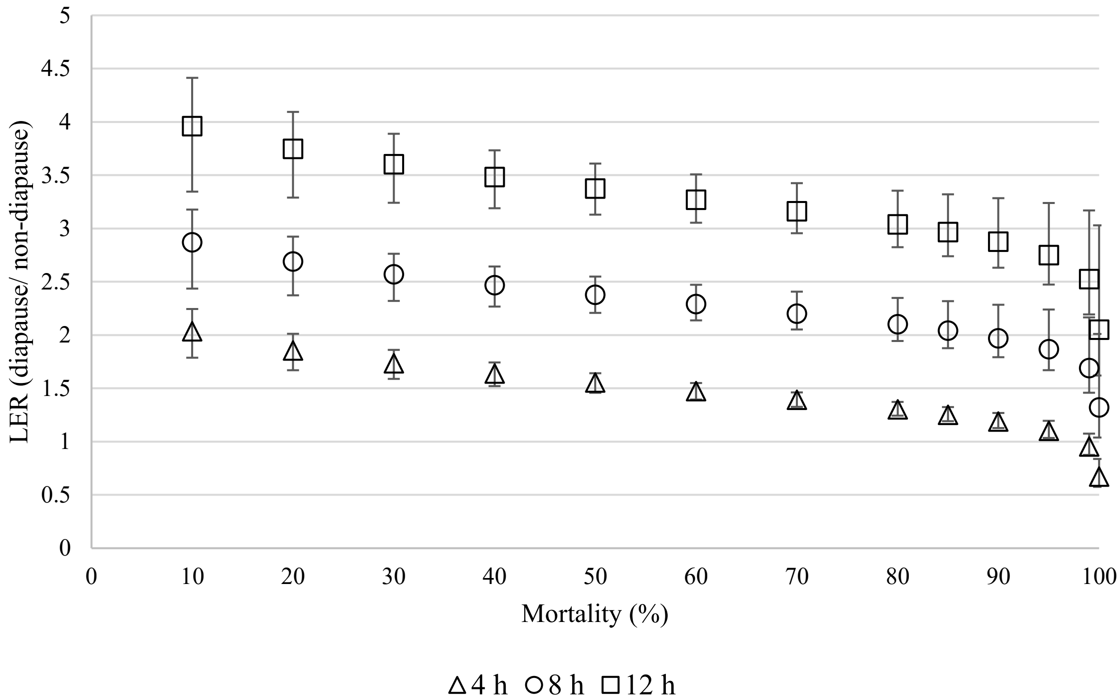


Figure 5.8. Relative susceptibility of adult *H. halys*, diapausing vs. non-diapausing.

Lethal exposure ratios (LERs) were calculated with (±) 95% confidence intervals for exposures projected, $(Ct)_p$, to cause 10 to 99.9968% (P9) mortality and used to identify that diapausing adult *H. halys* are more tolerant to eFUME^{TM} fumigation (16.7% by mass ethyl formate dilution in carbon dioxide) at relatively short durtations, while non-diapausing adult *H. halys* are more tolerant at relatively long durations. Relative to non-diapausing specimens, diapause resulted in a 0.7-, 1.3-, and 2.1-fold tolerance at 99.9968%, respective to the 4-, 8-, and 12-h fumigation durations.

5.6. Supplemental information

Table S5.1. Summary of female reproductive development rankings and statistical tests. Results of post-fumigation dissections of non-treated adult female *H. halys* control specimens are listed below. Mean cross-sectional ovary and spermatheca areas, ovary development score and spermatheca development score are shown for both diapause (D) and non-diapause (ND) insects treated under each of three fumigation durations in Table 5.2A; for all measures, the difference between females in "diapause" versus "non-diapause" was significant at a 95% confidence level ($P < 0.05$). Results of statistical tests are shown in Table 5.2B where "Score" (U) is the result of the Wilcoxon rank sum test, and "Area" is the result of the Student's t-test.

Table S5.3A								
	Ovary score		Ovary area (mm^2)		Spermatheca score		Spermatheca area (mm$^{2)}$	
Duration	**D**	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**

4 hours	1	2.38	4.22	21.19	1	1.96	1.56	3.48
8 hours	1	2.05	4.34	16.49	1	1.64	1.68	3.93
12 hours	1	2.44	4.80	16.58	1	2.00	1.65	3.94

Table S5.3B			
	Ovaries		
Duration	**Score**	**Area**	**Score**
4 hours	$U = 84$ ($P < 4.51 \times 10^{-9}$)	$t = -7.85$ ($P < 2.22 \times 10^{-8}$)	$U = 37.5$ ($P < 5 \times 10^{-10}$)
8 hours	$U = 42$ ($P < 1.25 \times 10^{-4}$)	$t = -4.24$ ($P < 0.00524$)	$U = 40$ ($P < 7.49 \times 10^{-5}$)
12 hours	$U = 50$ ($P < 7.28 \times 10^{-6}$)	$t = -4.789$ ($P < 2.14 \times 10^{-4}$)	$U = 40$ ($P < 1.95 \times 10^{-6}$)
	Spermatheca		
Duration	**Area**		
4 hours	$t = -6.54$ ($P < 1.27 \times 10^{-6}$)		
8 hours	$t = -5.84$ ($P < 1.27 \times 10^{-4}$)		
12 hours	$t = -5.79$ ($P < 2.1 \times 10^{-5}$)		

Table S5.2. Mortality of non-diapausing *H. halys* adults at ca. 10.0 °C.

The measured ethyl formate exposure ($(Ct)_m$), number of non-diapausing *H. halys* adults (n), and the observed mortality responses (r) following a 4-, 8-, or 12-h fumigation with an applied dose, C_0 (mgL^{-1}), of ethyl formate with an air temperature of ≥ 10.0 (±0.5) ºC ($\bar{x} \pm 2s$) are listed below. Ethyl formate was applied as a16.7% by dilution in carbon dioxide, consistent with the commercial formulation, eFUME™. Horizontal lines segment "fumigation blocks" conducted on different days.

Table S5.2

4-h duration (t = 4)				8-h duration (t = 8)				12-h duration (t= 12)			
C_0 (mgL^{-1})	Ct_m (mgL^{-1} h^{-1})	n	r	C_0 (mgL^{-1})	Ct_m (mgL^{-1} h^{-1})	n	r	C_0 (mgL^{-1})	Ct_m (mgL^{-1} h^{-1})	n	r
0	0	42	0	0	0	64	14	0	0	43	7
1.94	3.10	39	5	0.32	3.70	51	5	1.94	9.91	39	37
3.89	8.49	40	22	0.65	4.46	51	4	3.89	28.75	40	40
8.09	24.40	42	41	0.97	5.68	50	5	6.15	49.41	41	41
10.04	29.59	40	40	1.30	8.00	48	4	8.09	62.83	37	37
11.98	39.11	39	39	1.62	9.46	54	15	10.0	80.41	41	41
0	0	39	2	2.27	11.73	41	26	12.0	101.73	39	39
2.91	4.46	38	5	0	0	40	4	0	0	32	8
4.86	12.03	41	39	1.94	9.42	40	32	0.32	1.66	40	15
6.15	17.50	42	42	3.89	18.72	41	41	0.65	4.43	40	10
7.12	20.82	38	38	6.15	34.68	40	40	0.97	5.84	29	5
9.07	27.76	40	40	8.09	48.94	41	41	1.30	7.75	40	10
11.01	32.69	39	39	10.04	54.20	39	39	1.62	9.80	40	18
0	0	27	0	11.98	68.85	41	41	1.94	10.73	39	18
2.59	5.94	41	13	0	0	39	8	0	0	49	17
3.24	8.27	41	19	0.32	0.35	41	4	0.32	3.97	51	15

3.56	9.49	35	26	0.65	1.45	43	7	0.65	5.28	50	16
3.89	10.27	38	30	0.97	2.48	43	6	0.98	7.37	50	12
4.53	10.77	32	28	1.30	3.14	42	6	3.3	14.45	49	49
0	0	48	5	1.62	4.23	40	5	4.87	32.90	50	50
0.32	0.81	50	8	1.94	9.43	41	36	9.76	82.11	49	49
0.65	1.83	50	9	0	0	43	4	0	0	49	9
0.97	2.98	49	17	0.32	3.92	42	4	0.98	8.51	49	8
1.30	3.79	51	7	0.65	4.90	48	8	1.62	10.39	49	34
1.62	4.93	47	10	0.97	6.15	41	5	1.95	15.44	50	33
1.94	5.95	49	9	1.30	6.49	40	6	3.58	21.56	50	49
0	0	52	4	1.62	7.96	39	16	4.22	32.46	49	49
4.21	9.86	52	14	1.94	9.54	39	25	5.19	43.16	51	51
4.53	10.96	51	22					0	0	40	6
5.18	14.11	51	26					0.32	0.51	41	2
5.50	14.96	51	46					0.65	2.12	38	2
6.15	17.85	50	48					0.97	3.43	41	4
6.48	20.14	50	50					1.30	4.89	39	0
								1.62	11.90	40	40
								1.94	13.16	43	43

Table S5.3. Mortality of diapausing *H. halys* adults at ca. 10.0 °C.

Measured exposure ($(Ct)_{m.}$), number (n) of diapausing (D) *H. halys* adults, the number (n) of non-diapausing (ND) *H. halys* adults included for direct comparison to a given exposure, and the respective observed mortality responses (r) following a 4-, 8-, or 12-h fumigation with an applied dose, C_0 (mgL^{-1}), of ethyl formate with air temperature ≥ 10.0 (±0.5) ºC ($\bar{x} \pm 2s$) are listed below. Ethyl formate was applied as a 16.7% by dilution in carbon dioxide, consistent with the commercial formulation, eFUME™. Horizontal lines segment "fumigation blocks" conducted on different days. Table S5.2 has been divided into Table S5.3A, B, and C.

Table S5.3A

4 - h duration (t = 4 h)					4 - h duration (t = 4 h)				
	C_0 (mgL^{-1})	Ct_m (mgL^{-1} h^{-1})	n	r		C_0 (mgL^{-1})	Ct_m (mgL^{-1} h^{-1})	n	r
D	0	0	30	1	D	0	0	30	4
D	9.39	37.23	30	30	D	7.45	28.55	30	30
D	9.39	38.04	31	31	D	7.45	29.16	31	31
D	9.39	37.65	30	30	D	7.45	28.65	29	29
D	5.5	19.93	30	27	D	6.48	22.01	29	29
D	5.5	19.47	29	25	D	6.48	21.97	29	28
D	5.5	20.49	30	19	D	6.48	22.7	30	29
ND	0	0	22	12	ND	0	0	20	1
ND	9.39	37.23	19	19	ND	7.45	28.55	20	20
ND	9.39	38.04	20	20	ND	7.45	29.16	19	19
ND	9.39	37.65	19	19	ND	7.45	28.65	22	22
ND	5.5	19.93	20	18	ND	6.48	22.01	22	22
ND	5.5	19.47	21	21	ND	6.48	21.97	21	21
ND	5.5	20.49	22	22	ND	6.48	22.7	19	19
D	0	0	26	2	D	0	0	31	2
D	3.89	14.52	25	0	D	10.04	36.39	30	30
D	3.89	13.14	28	3	D	10.04	35.38	30	30
D	3.89	15.6	28	4	D	10.04	36.56	30	30
D	5.5	20.51	25	18	D	9.39	33.5	31	30
D	5.5	20.73	26	23	D	9.39	33.31	30	30
D	5.5	21.56	28	21	D	9.39	33.34	29	29
ND	0	0	23	7	ND	0	0	20	2
ND	3.89	14.52	22	14	ND	10.04	36.39	21	21

ND	3.89	13.14	22	12	ND	10.04	35.38	19	19
ND	3.89	15.6	19	14	ND	10.04	36.56	21	21
ND	5.5	20.51	23	21	ND	9.39	33.5	21	21
ND	5.5	20.73	18	17	ND	9.39	33.31	19	18
ND	5.5	21.56	21	21	ND	9.39	33.34	20	20
D	0	0	32	2	D	0	0	33	0
D	10.04	43.38	32	32	D	1.94	4.44	33	1
D	10.04	41.61	29	29	D	4.21	11.38	30	7
D	10.04	43.82	32	32	D	6.15	16.78	31	25
D	9.39	39.83	30	30	D	8.09	24.14	33	32
D	9.39	39.22	29	29	D	10.04	29.73	31	31
D	9.39	39.21	40	40	D	11.98	36.38	36	36
ND	0	0	22	6	ND	0	0	21	3
ND	10.04	43.38	19	18	ND	1.94	4.44	25	2
ND	10.04	41.61	19	19	ND	4.21	11.38	19	6
ND	10.04	43.82	20	20	ND	6.15	16.78	22	18
ND	9.39	39.83	21	21	ND	8.09	24.14	18	18
ND	9.39	39.22	20	20	ND	10.04	29.73	21	21
ND	9.39	39.21	21	21	ND	11.98	36.38	23	23

Table S5.3B Continued from Table S5.3A

4 - h duration (t = 4 h)					4 - h duration (t = 4 h)				
	C_0 (mgL^{-1})	Ct_m (mgL^{-1} h^{-1})	n	r		C_0 (mgL^{-1})	Ct_m (mgL^{-1} h^{-1})	n	r
D	0	0	32	5	D	0	0	30	0
D	6.48	25.44	34	33	D	2.59	5.23	30	1
D	6.48	25.38	30	28	D	4.53	11.52	28	1
D	6.48	24.63	33	31	D	6.48	18.86	30	26
D	3.89	13.76	32	0	D	8.74	25.07	30	30
D	3.89	13.94	29	11	D	10.69	31.25	29	29
D	3.89	13.86	31	12	D	12.63	38.14	29	29
ND	0	0	19	8	ND	0	0	20	6
ND	6.48	25.44	20	18	ND	2.59	5.23	20	1
ND	6.48	25.38	20	20	ND	4.53	11.52	20	9
ND	6.48	24.63	22	21	ND	6.48	18.86	20	19
ND	3.89	13.76	21	16	ND	8.74	25.07	20	20
ND	3.89	13.94	19	13	ND	10.69	31.25	20	20
ND	3.89	13.86	21	17	ND	12.63	38.14	21	21
D	0	0	30	1	D	0	0	28	0
D	4.86	20.93	31	23	D	4.53	14.05	31	4
D	4.86	18.38	30	21	D	5.5	16.43	31	22
D	4.86	21.89	30	26	D	6.48	20.45	32	28
D	6.48	26.82	30	30	D	7.12	21.67	34	31
D	6.48	28.06	30	30	D	8.09	25.73	31	29
D	6.48	28.96	31	30	D	8.74	27.82	29	29
ND	0	0	19	7	ND	0	0	20	1
ND	4.86	20.93	21	21	ND	4.53	14.05	22	9
ND	4.86	18.38	21	20	ND	5.5	16.43	21	13
ND	4.86	21.89	20	18	ND	6.48	20.45	20	20
ND	6.48	26.82	20	20	ND	7.12	21.67	20	19
ND	6.48	28.06	20	19	ND	8.09	25.73	19	19
ND	6.48	28.96	19	18	ND	8.74	27.82	22	22

Table S5.3C Continued from Table S5.3B									
8 - h duration (t = 8 h)					12 - h duration (t= 12 h)				
	C_0 (mgL^{-})	Ct_m (mgL^{-1} h^{-1})	n	r		C_0 (mgL^{-})	Ct_m (mgL^{-1} h^{-1})	n	r
D	0	0	33	0	D	0	0	29	3
D	2.59	10.29	32	0	D	0.32	1.29	29	0
D	4.53	21.75	10	10	D	0.65	5.97	28	0
D	5.50	30.41	32	31	D	0.97	11.18	30	0
D	6.48	34.82	30	30	D	1.94	22.26	30	4
D	8.74	49.09	31	31	D	3.89	47.19	30	23
D	10.69	63.46	30	30	D	6.15	59.81	31	31
ND	0	0	20	1	ND	0	0	20	2
ND	2.59	10.29	21	3	ND	0.32	1.29	20	7
ND	4.53	21.75	21	21	ND	0.65	5.97	20	4
ND	5.50	30.41	20	20	ND	0.97	11.18	20	10
ND	6.48	34.82	21	21	ND	1.94	22.26	19	17
ND	8.74	49.09	20	20	ND	3.89	47.19	22	22
ND	10.69	63.46	20	20	ND	6.15	59.81	20	20
D	0	0	30	0	D	0	0	33	1
D	0.97	2.67	29	4	D	1.94	11.99	31	3
D	2.27	11.23	30	1	D	2.27	13.71	30	1
D	2.59	12.76	30	2	D	2.59	18.68	31	0
D	4.53	26.78	29	11	D	3.24	21.35	31	0
D	6.80	41.37	29	26	D	3.56	24.18	30	7
D	8.74	50.43	29	29	D	4.53	31.14	30	18
ND	0	0	20	5	ND	0	0	20	5
ND	0.97	2.67	19	3	ND	1.94	11.99	20	3
ND	2.27	11.23	21	10	ND	2.27	13.71	21	7
ND	2.59	12.76	20	14	ND	2.59	18.68	20	20
ND	4.53	26.78	19	19	ND	3.24	21.35	20	20
ND	6.80	41.37	20	20	ND	3.56	24.18	20	20
ND	8.74	50.43	19	19	ND	4.53	31.14	20	20
D	0	0	20	2	D	0	0	31	0
D	1.94	7.87	19	0	D	3.56	30.90	30	12
D	2.59	13.76	20	3	D	4.53	37.48	31	29
D	3.56	20.77	20	3	D	5.50	50.34	29	25
D	3.89	21.04	20	5	D	6.48	55.98	29	29
D	4.21	23.54	20	8	D	7.77	66.76	30	30
D	4.53	26.57	20	6	D	8.74	80.37	35	35
ND	0	0	21	4	ND	0	0	20	2
ND	1.94	7.87	17	2	ND	3.56	30.90	20	20
ND	2.59	13.76	19	6	ND	4.53	37.48	20	20
ND	3.56	20.77	19	19	ND	5.50	50.34	18	18
ND	3.89	21.04	20	20	ND	6.48	55.98	20	20
ND	4.21	23.54	19	19	ND	7.77	66.76	22	22
ND	4.53	26.57	20	20	ND	8.74	80.37	19	19

Chapter 6: Conclusions

Global trade is dependent on the responsible transportation of goods in cooperation with National Plant Protection Organizations to prevent the accidental transportation of insect hitchhikers, which may be dangerous to agriculture, economy, and, most importantly, ecosystem. Postharvest fumigation is the most common method of controlling insect hitchhikers, but it can also be paired with IPM control elements.

Blueberry maggot is a regulatory trade barrier for fresh blueberries originating from Eastern North American blueberry growers. To facilitate access, we developed a systems-based approach that incorporates existing IPM strategies and postharvest fumigation with MB. The effect of current IPM strategies was quantified by comparing the relative numbers of pupae reared from managed and unmanaged blueberry orchards in Southwest Michigan. The MB fumigation required a new method to quantify mortality when using field-infested blueberries. We found that our rearing methods were statistically the same as BBM reared in blueberries on the bush. After developing methods for identifying immature BBM life stages retrospectively, the application of the MB schedule required for SWD control yielded sufficient control of BBM to be statistically combined with IPM control elements to achieve an overall probit 9 mortality.

Brown marmorated stink bug has already spread from Asia to every Northern Hemisphere continent, and while no established populations had been detected in the Southern Hemisphere as of 2017, all southern hemisphere countries are at risk.[21] The development of an ethyl formate and carbon dioxide fumigation provides a means for non-horticultural goods infested with BMSB to be safely transported from the US, with minimal impact to the environment.

References

1 Follett PA and Neven LG, Current trends in quarantine entomology, *Annu Rev Entomol* **51**:359–385 (2006).

2 Kramer J, Simnitt S, and Calvin L, Fruit and Tree Nuts Outlook : September 2020, *United States Dep Agric* **FTS-371**:1–45 (2020).

3 Argentina prepares for the 2020 blueberry season, *Blueberries Consult Mag*, 2021. https://blueberriesconsulting.com/en/argentina-se-prepara-para-la-temporada-de-arandanos-2020/#:~:text=shipments.,blueberries as a niche actor. [accessed 10 August 2021].

4 Crop and Export Report 2021, Week 12, *Chil Blueberry Comm*, 2021. https://www.producereport.com/article/chilean-blueberry-exports-rise-peak-season-arrives [accessed 10 August 2021].

5 Food and Agriculture Organization of the United Nations, FAOSTAT statistical database, United Nations, Rome (1997).

6 Argentine blueberry production struggles to remain profitable, *Fresh Plaza*, 2021. https://www.freshplaza.com/article/9342685/argentine-blueberry-production-struggles-to-remain-profitable/#:~:text=According to a study prepared,by sea to remain profitable. [accessed 10 August 2021].

7 Karst T, Marketers expect good season for Chilean blueberries, *Pack*, 2021. https://www.thepacker.com/markets-news/produce-crops-markets/shipping/marketers-expect-good-season-chilean-blueberries#:~:text=Total Chilean blueberry exports

to,%24110 million from 2017-18. [accessed 10 August 2021].

8 Retamales JB, Palma MJ, Morales YA, Lobos GA, Moggia CE, and Mena CA, Produção de mirtilo no Chile: Situação atual e desenvolvimentos futuros, *Rev Bras Frutic* **36**:58–67 (2014).

9 Bachmann J and Earles R, Postharvest handling of fruits and vegetables, *Appropr Technol Transf Rural Areas*, 1–19 (2000).

10 Boyette M, Estes E, Mainland CM, and Cline B, Postharvest cooling and handling of blueberries, *North Carolina State Ext* **AG-413-07**:413–415 (1993).

11 Almenar E, Samsudin H, Auras R, Harte B, and Rubino M, Postharvest shelf life extension of blueberries using a biodegradable package, *Food Chem* **110**:120–127 (2008).

12 Harb JY and Streif J, Controlled atmosphere storage of highbush blueberries cv. "Duke," *Eur J Hortic Sci* **69**:66–72 (2004).

13 Chiabrando V and Giacalone G, Studies on shelf life of "Lateblue" highbush blueberries, *Food Sci Technol Int* **14**:199–205 (2008).

14 Rosenfeld HJ, Røed Meberg K, Haffner K, and Sundell HA, MAP of highbush blueberries: Sensory quality in relation to storage temperature, film type and initial high oxygen atmosphere, *Postharvest Biol Technol* **16**:27–36 (1999).

15 Concha-Meyer A, Eifert JD, Williams RC, Marcy JE, and Welbaum GE, Shelf life determination of fresh blueberries (vaccinium corymbosum) stored under controlled atmosphere and ozone, *Int J Food Sci* **2015**:1–9 (2015).

16 International Trade Administration, US Department of Commerce, Washington, D.C.

(2021).

17 Rodriguez-Saona C, Vincent C, and Isaacs R, Blueberry IPM: past successes and future challenges, *Annu Rev Entomol* **64**:73–93 (2018).

18 Lathrop FH and Nickels CB, The biology and control of the blueberry maggot in Washington County, Maine., *United States Dep Agric Techinical Bull* **275**:1–77 (1932).

19 Rodriguez-Saona C, Vincent C, Polk D, and Drummond FA, A review of the blueberry maggot fly (Diptera: Tephritidae), *J Integr Pest Manag* **15**:11–21 (2015).

20 Faraone N, Hillier NK, and Cutler GC, Collection of host-marking pheromone from Rhagoletis mendax (Diptera: Tephritidae), *Can Entomol* **148**:552–555 (2016).

21 Kriticos DJ, Kean JM, Phillips CB, Senay SD, Acosta H, and Haye T, The potential global distribution of the brown marmorated stink bug, Halyomorpha halys, a critical threat to plant biosecurity, *J Pest Sci (2004)* **90**:1033–1043 (2017).

22 Stern VM, Smith RF, van den Bosch R, and Hagen KS, The integrated control concept, *J Agric Sci* **29**:81–101 (1959).

23 Katagi T, Environmental behavior of synthetic pyrethroids, ed. by Matsuo N and Mori T, Pyrethroids: From Chrysanthemum to Modern Industrial Insecticide, Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 167–202 (2012).

24 Khrimian A, Zhang A, Weber DC, Ho HY, Aldrich JR, Vermillion KE, *et al.*, Discovery of the aggregation pheromone of the brown marmorated stink bug (Halyomorpha halys) through the creation of stereoisomeric libraries of 1-bisabolen-3-ols, *J Nat Prod* **77**:1708–1717 (2014).

25 Leskey TC, Khrimian A, Weber DC, Aldrich JC, Short BD, Lee DH, *et al.*, Behavioral responses of the invasive *Halyomorpha halys* (Stål) to traps baited with stereoisomeric mixtures of 10,11-epoxy-1-bisabolen-3-OL, *J Chem Ecol* **41**:418–429 (2015).

26 Weber DC, Morrison WR, Khrimian A, Rice KB, Short BD, Herlihy M V., *et al.*, Attractiveness of pheromone components with and without the synergist, methyl (2E,4E,6Z)-2,4,6-decatrienoate, to brown marmorated stink bug (Hemiptera: Pentatomidae), *J Econ Entomol* **113**:712–719 (2020).

27 Animal and Plant Health Inspection Service, Treatment manual, 2nd ed., *United States Dep Agric*, 2nd ed. (2016).

28 Monro HAU, Manual of fumigation for insect control. Second edition, revised., *F A O Agric Stud* (1984).

29 Montreal protocol on substances that deplete the ozone layer, United Nations, Ozone Secretariat, Nairobi, Kenya, p. 960 (1987).

30 Walse SS, Leesch JG, and Tebbets S, (2009).

31 L.Yee W, Chapman PS, and K.Tanigoshi L, Alternative fumigants to methyl bromide for killing pupae and preventing emergence of apple maggot fly (Diptera: Tephritidae), *J Entomol Sci* **48**:36–42 (2013).

32 Ryan R. F and De Lima CPF, Ethyl formate fumigation an overview update, *11th Int Work Conf Stored Prod Prot*, 969–978, 2014 Nov 24-28, Chiang Mai, Thailand (2014).

33 Hansen JD, Hara AH, Chan Jr. HT, and Tenbrink VL, Efficacy of hydrogen cyanide fumigation as a treatment for pests of hawaiian cut flowers and foliage after harvest, *J*

Econ Entomol **84**:532–536 (1991).

34 Indirect food substances affirmed as generally recognized as safe 21 C.F.R. § 186, (1977).

35 Lee SJ, Mehler L, Beckman J, Diebolt-Brown B, Prado J, Lackovic M, *et al.*, Acute pesticide illnesses associated with off-target pesticide drift from agricultural applications: 11 states, 1998-2006, *Environ Health Perspect* **119**:1162–1169 (2011).

36 Guedes RNC, Walse SS, and Throne JE, Sublethal exposure, insecticide resistance, and community stress, *Curr Opin Insect Sci* **21**:47–53 (2017).

37 Relyea RA, The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities, *Ecol Appl* **15**:618–627 (2005).

38 Stark JD and Banks JE, Population-Level Effects of Pesticides and Other Toxicants on Arthropods, *Annu Rev Entomol* **48**:505–519 (2003).

39 Bonmatin JM, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, *et al.*, Environmental fate and exposure; neonicotinoids and fipronil, *Environ Sci Pollut Res* **22**:35–67 (2015).

40 Kamel F and Hoppin JA, Association of pesticide exposure with neurologic dysfunction and disease, *Environ Health Perspect* **112**:950–958 (2004).

41 Eskenazi B, Marks AR, Bradman A, Harley K, Barr DB, Johnson C, *et al.*, Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children, *Environ Health Perspect* **115**:792–798 (2007).

42 Damalas CA and Eleftherohorinos IG, Pesticide exposure, safety issues, and risk assessment indicators, *Int J Environ Res Public Health* **8**:1402–1419 (2011).

43 ISPM 28, Phytosanitary treatments for regulated pests (2007).

44 Jang EB, Mau RFL, Vargas RI, and Mcinnis DO, Exporting fruit from low fruit fly prevalence zones with multiple mitigation systems approach, *Proc Int Symp Areawide Manag Insect Pests*, 63–69, 2006 Oct 1-5, Okinawa, Japan.

45 ISPM 14, The use of integrated measures in a systems approach for pest risk managmeent (2017).

46 Vail P V., Tebbets JS, Mackey BE, and Curtis CE, Quarantine treatments: A biological approach to decision-making for selected hosts of codling moth (Lepidoptera: Tortricidae), *J Econ Entomol* **86**:70–75 (1993).

47 Jang EB and Moffitt HR, Systems approaches to achieving quarantine security, ed. by Sharp JL and Hallman GJ, Quarantine Treatments for Pests of Food Plants, Avalon Books, New York, NY, pp. 225–237 (1994).

48 Jang EB, Systems approach to quarantine security: Postharvest application of sequential mortality in the hawaiian grown "Sharwil" avocado system, *J Econ Entomol* **89**:950–956 (1996).

49 Weaver DK, Opit GP, Mason LJ, and Throne JE, Gravimetric method for determining stage of an obligate internally feeding stored-product insect pest, the cowpea weevil, *Environ Entomol* **35**:1483–1490 (2006).

50 Dobie P, An investigation into the use of an X-ray technique in the study of pre-emergent stages of Sitophilus oryzae (L.) developing in Manitoba wheat, *J Stored Prod Res* **9**:7–12 (1973).

51 Shade RE, Furgason ES, and Murdock LL, Detection of Hidden Insect Infestations by Feeding-Generated Ultrasonic Signals, *Am Entomol* **36**:231–235 (1990).

52 Hsin Chi, Life-table analysis incorporating both sexes and variable development rates among individuals, *Environ Entomol* **17**:26–34 (1988).

53 Bellamy DE and Walse SS, Distribution of spotted wing drosophila life stages, 2013. https://www.ars.usda.gov/pacific-west-area/parlier/sjvasc/cpq/docs/spotted-wing-drosophila-development/services/ [accessed 2 August 2019].

54 Neilson WTA and Mcallan JW, Artificial diets for the apple Maggot. III. Improved, defined diets, *J Econ Entomol* **58**:542–543 (1965).

55 Manly BFJ, A review of methods for the analysis of stage-frequency data, *Estim Anal Insect Popul*, ed. by McDonald LL, Manly BFJ, Lockwood JA, and Logan JA, 3–69, 1988 Jan 25-29, New York, NY.

56 Skoog Leary, James J., DA, Principles of instrumental analysis, Saunders College Pub., Fort Worth, TX (1992).

57 Bellamy DE, Sisterson MS, and Walse SS, Quantifying host potentials: indexing postharvest fresh fruits for spotted wing drosophila, Drosophila suzukii, *PLoS One* **8**:e61227 (2013).

58 Melampy RM and Willis ER, Respiratory metabolism during larval and pupal development of the female honeybee (*Apis mellifica L.*), *Physiol Zool* **12**:302–311 (1939).

59 O'Donnell A, Determination of larval instars of the rice weevil Sitophilus oryzae (L.) (Coleoptera: Curculionidae) in wheat, *J Ark Acad Sci* **21**, 36–38 (1967).

60 Bellows TS and Birley MH, Estimating developmental and mortality rates and stage recruitment from insect stage-frequency data, *Res Popul Ecol (Kyoto)* **23**:232–244 (1981).

61 Richards CS and Villet MH, Factors affecting accuracy and precision of thermal summation models of insect development used to estimate post-mortem intervals, *Int J Legal Med* **122**:401–408 (2008).

62 Baqué M and Amendt J, Strengthen forensic entomology in court-the need for data exploration and the validation of a generalised additive mixed model, *Int J Legal Med* **127**:213–223 (2013).

63 Amendt J, Goff ML, Campobasso CP, and Grassberger M, Current concepts in forensic entomology, *Curr Concepts Forensic Entomol* (2010).

64 Von Zuben CJ, Bassanezi RC, and Von Zuben FJ, Theoretical approaches to forensic entomology: II. Mathematical model of larval development, *J Appl Entomol* **122**:275–278 (1998).

65 Bánki O, Roskov Y, Vandepitte L, DeWalt RE, Remsen D, Schalk P, *et al.*, Catalogue of Life Checklist, Catalogue of Life, Leiden, Netherlands (2021).

66 Aliniazee MT and Long LE, Biology and control of the cherry fruit flies : A worldwide perspective, *Entomol Exp Appl*, ed. by Aliniazee MT and Long LE, 5–8, 1996 Mar 3, The Dalles, Oregon.

67 Emery SE and Mills NJ, Effects of temperature and other environmental factors on the post-diapause development of walnut husk fly Rhagoletis completa (Diptera: Tephritidae), *Physiol Entomol* **44**:33–42 (2019).

68 Compton JM, Cowgill WP, and Tietjen WH, A review of cherry fruit fly: The eastern USA perspective, *Acta Hortic* **667**:489–494 (2005).

69 Finney DJ, The application of the probit method to toxicity test data adjusted for mortality in the controls, *Ann Appl Biol* **31**:68–74 (1944).

70 Ehlenfeldt MK, Meredith FI, and Ballington JR, Unique organic acid profile of rabbiteye vs. highbush blueberries, *HortScience* **29**:321–323 (1994).

71 VINCENT C, LEMOYNE P, GAUL S, and MACKENZIE K, Factors limiting the northern distribution of the blueberry maggot, *Rhagoletis mendax* (Diptera: Tephritidae) in Eastern Canada, *Eur J Entomol* **113**:143–149 (2016).

72 Régnier B, Legrand J, and Rebaudo F, Modeling temperature-dependent development rate in insects and implications of experimental design, ed. by Brent C, *Environ Entomol* **51**:132–144 (2022).

73 R Core Team, R: A Language and Environment for Statistical Computing, Vienna, Austria (2013).

74 RStudio Team, RStudio: Integrated Development Environment for R, Boston, MA (2020).

75 Teixeira LAF and Polavarapu S, Postdiapause development and prediction of emergence of female blueberry maggot (Diptera: Tephritidae), *Environ Entomol* **30**:925–931 (2001).

76 Wise JC, Gut LJ, Wilson J, Greishop M, Mota-Sanchez D, Quintanilla M, *et al.*, Fruit pest management guide (2021), Michigan State University Extension, East Lansing, Michigan (2021).

77 Tebbets JS and Walse SS, Postharvest treatment of blueberries with methyl bromide to

control spotted wing drosophila, Drosophila suzukii. Report for California Blueberry Commission, United States High Bush Blueberry Commission, USDA-APHIS, and Australia. (2014).

78 Roth H and Richardson HH, Ethylene dibromide, methyl bromide, and ethylene chlorobromide as fumigants for control of apple and blueberry maggots in fruit, *J Econ Entomol* **63**:496–499 (1970).

79 Melvin Couey H and Chew V, Confidence limits and sample size in quarantine research, *J Econ Entomol* **79**:887–890 (1986).

80 Armbruster DA and Pry T, Limit of blank, limit of detection and limit of quantitation., *Clin Biochem Rev* **29 Suppl 1**:S49-52 (2008).

81 USDA, Fumigation and chemistry group of the commodity protection and quality research unit, 2010. http://www.ars.usda.gov//Main/site_main.htm?docid=18577 [accessed 2 August 2019].

82 Finney DJ, Probit analysis, 3rd ed., *Cambride Univ Press*, 3rd ed., Cambridge University Press (1971).

83 Liquido NJ and Griffin RL, Quarantine treatment statistics. United States Department of Agriculture, *Cent Plant Heal Sci Technol Raleigh, NC Available from http//cqtstats cphst org/index cfm* (2010).

84 Darby JA, A kinetic model of fumigant sorption by grain using batch experimental data, *Pest Manag Sci* **64**:519–526 (2008).

85 Walse SS, Myers SW, Liu Y-B, Bellamy DE, Obenland D, Simmons GS, *et al.*,

Postharvest treatment of fresh fruit from california with methyl bromide for control of light brown apple moth (Lepidoptera: Tortricidae), *J Econ Entomol* **106**:1155–1163 (2013).

86 Walse SS, Tebbets JS, and Leesch JG, Postharvest methyl bromide fumigation of Japanese plums to control codling moth (Lepidoptera: Tortricidae), *J Asia Pac Entomol* **22**:807–815, Elsevier (2019).

87 Tebbets JS and Walse SS, Postharvest treatment of blueberries with methyl bromide to control spotted wing drosophila , Drosophila suzukii, 1–7 (2014).

88 Ortiz CM, Franceschinis F, Gergoff Grozeff GE, Chan H, Labavitch JM, Crisosto C, *et al.*, Pre-treatment with 1-methylcyclopropene alleviates methyl bromide-induced internal breakdown, softening and wall degradation in blueberry, *Postharvest Biol Technol* **146**:90–98 (2018).

89 Thang K, Au K, Rakovski C, and Prakash A, Effect of phytosanitary irradiation and methyl bromide fumigation on the physical, sensory, and microbiological quality of blueberries and sweet cherries, *J Sci Food Agric* **96**:4382–4389 (2016).

90 Lan Eum H, Chul Hong S, Chun C, Sheob Shin I, Yil Lee B, Ki Kim H, *et al.*, Influence of temperature during transport on shelf-life quality of highbush blueberries (*Vaccinium corymbosum* L. cvs. Bluetta, Duke), *Hort Environ Biotechnol* **54**:128–133 (2013).

91 Rosenthal R, Combining results of independent studies., *Psychol Bull* **85**:185–193 (1978).

92 Leskey TC, Hamilton GC, Nielsen AL, Polk DF, Rodriguez-Saona C, Christopher Bergh J, *et al.*, Pest status of the brown marmorated stink bug, *Halyomorpha halys* in the USA,

Outlooks Pest Manag **23**:218–226 (2012).

93 Abrams AE, Kawagoe JC, Najar-Rodriguez A, and Walse SS, Sulfuryl fluoride fumigation to control brown marmorated stinkbug (Hempitera: Pentatomidae), *Postharvest Biol Technol* **163**:111111 (2020).

94 Lee D-H, Cullum JP, Anderson JL, Daugherty JL, Beckett LM, and Leskey TC, Characterization of overwintering sites of the invasive brown marmorated stink bug in natural landscapes using human surveyors and detector canines, *PLoS One* **9**:1–9 (2014).

95 Inkley DB, Characteristics of home invasion by the brown marmorated stink Bug (Hemiptera: Pentatomidae), *J Entomol Sci* **47**:125–130 (2012).

96 Australian Department of Agriculture and Water Resources, Draft pest risk analysis for brown marmorated stink bug (*Halyomorpha halys*) (2017).

97 Ministry for Primary Industries, Treatment requirement: Approved biosecurity treatments (2018).

98 Bond EJ, Manual of fumigation for insect control: FAO plant production and protection paper, FAO (1984).

99 US Department of Commerce ITA, Trends in U.S. vehicle exports, 15 (2015).

100 Neifert IE, Cook FC, Roark RC, Tonkin WH, Back EA, and Cotton RT, Fumigation against grain weevils with various volatile organic compounds, *United States Dep Agric Techinical Bull*:1–40 (1925).

101 Armstrong JW, Fruit fly disinfestation strategies beyond methyl bromide, *New Zeal J Crop Hortic Sci* **20**:181–193 (1992).

102 Kim K, Lee YH, Kim G, Lee B-H, Yang J-O, and Lee S-E, Ethyl formate and phosphine fumigations on the two-spotted spider mite, Tetranychus urticae and their biochemical responses, *Appl Biol Chem* **62**:50 (2019).

103 Xin N, Ren YL, Forrester RI, Ming X, and Mahon D, Toxicity of ethyl formate to adult *Sitophilus oryzae* (L.), *Tribolium castaneum* (herbst) and *Rhyzopertha dominica* (F.), *J Stored Prod Res* **44**:241–246 (2008).

104 Bikoba VN, Pupin F, Biasi W V., Rutaganira FU, and Mitcham EJ, Use of ethyl formate fumigation to control adult bean thrips in navel oranges, *J Econ Entomol* **112**:591–596 (2019).

105 Simpson T, Bikoba V, and Mitcham EJ, Effects of ethyl formate on fruit quality and target pest mortality for harvested strawberries, *Postharvest Biol Technol* **34**:313–319 (2004).

106 Ryan RF and De Lima CPF, Fumigation of citrus using ethyl formate + carbon dioxide as a quarantine treatment, 2010, 34.

107 Graver JE V, Weller GL, and van S Graver JE, Cut flower disinfestation: Assessment of replacement fumigants for methyl bromide, *Postharvest Biol Technol* **14**:325–333 (1998).

108 Simpson T, Bikoba V, Tipping C, and Mitcham EJ, Ethyl formate as a postharvest fumigant for selected pests of table grapes, *J Econ Entomol* **100**:1084–1090 (2007).

109 Haritos VS and Dojchinov G, Cytochrome c oxidase inhibition in the rice weevil *Sitophilus oryzae* (L.) by formate, the toxic metabolite of volatile alkyl formates, *Comp Biochem Physiol - C Toxicol Pharmacol* **136**:135–143 (2003).

110 Haritos VS, Damcevski KA, and Dojchinov G, Improved efficacy of ethyl formate against

stored grain insects by combination with carbon dioxide in a “dynamic” application, *Pest Manag Sci* **62**:325–333 (2006).

111 Lee BH, Park CG, Park MG, Roh GH, Kim D, Riddick EW, *et al.*, Ethyl formate fumigation for the disinfestation of red imported fire ants Solenopsis invicta Buren, *J Asia Pac Entomol* **22**:838–840, Elsevier (2019).

112 Ren Y, Lee B, Padovan B, and Cai L, Ethyl formate plus methyl isothiocyanate-a potential liquid fumigant for stored grains, *Pest Manag Sci* **68**:194–201 (2012).

113 Coetzee EM, Du X, Thomas ML, Ren Y, and McKirdy SJ, In-transit fumigation of shipping containers with ethyl formate + nitrogen on road and continued journey on sea, *J Environ Sci Heal Part B* **55**:820–826 (2020).

114 Zabetakis MG, Flammability characteristics of combustiple gases and vapors, *Bull 627*, U.S. Bureau of Mines, Washington, U.S. (1965).

115 Jones RM, Reducing inflammability of fumigants with carbon dioxide, *Ind Eng Chem* **25**:394–396 (1933).

116 Bond EJ and Buckland CT, Control of insects with fumigants at low temperatures: Toxicity of fumigants in atmospheres of carbon dioxide, *J Econ Entomol* **71**:307–309 (1978).

117 Ethyl formate 21 C.F.R. § 184.1295, (1984).

118 Abrams AE, Alvarez A, Rodriguez MS, Kron CR, Bellamy DE, and Walse SS, Greenhouse rearing methods for brown marmorated stink bug (Hemiptera: Pentatomidae) on live cowpea plants, *J Econ Entomol* **114**:2297–2306 (2021).

119 Abbott WS, A method of computing the effectiveness of an insecticide, *J Econ Entomol* **18**:265–267 (1925).

120 Quinn TJ, Temperature (monographs in physical measurement), Academic Press London LTD (1983).

121 Kim BS, Song JE, Park JS, Park YJ, Shin EM, and Yang JO, Insecticidal effects of fumigants (EF, MB, and PH3) towards phosphine-susceptible and –resistant sitophilus oryzae (coleoptera: Curculionidae), *Insects* **10**:327 (2019).

122 Vincest LE and Lindgren DL, Hydrogen phosphide and ethyl formate: Fumigation of insects infesting dates and other dried fruits, *J Econ Entomol* **65**:1667–1669 (1972).

123 Park M, Lee B, Yang J, Kim B, Roh GH, Kendra PE, *et al.*, Ethyl formate as a methyl bromide alternative for fumigation of citrus: Efficacy, fruit quality, and workplace safety, *J Econ Entomol* **114**:2290–2296 (2021).

124 Desmarchelier JM, Johnston FM, and Le Trang Vu, Ethyl formate, formic acid and ethanol in air, wheat, barley and sultanas: Analysis of natural levels and fumigant residues, *Pestic Sci* **55**:815–824 (1999).

125 Gautam S, Corbett S, Grafton-Cardwell E, and Walse SS, Commercial fumigation of fresh citrus with ethyl formate (Vapormate), *Proc Methyl Bromide Altern Outreach*, 39, 2019 Nov 11-13, San Diego, California (2019).

126 Mata-Segreda JF, Spontaneous hydrolysis of ethyl formate: Isobaric activation parameters, *Int J Chem Kinet* **32**:67–71 (2000).

127 Wolfram Research Inc, Mathematica, Wolfram Research Inc., Champaign, Illinois (2021).

128 Kawagoe JC, Abrams AE, and Walse SS, Ethyl formate fumigation for control of stinkbugs in vehicle consignments: The impact of diapause, *Proc Methyl Bromide Altern Outreach*, 2019 November 11-13, San Diego, California (2019).

129 Bliss CI, The relation between exposure time, concentration and toxicity in experiments on insecticides, *Ann Entomol Soc Am* **33**:721–766 (1940).

130 Miller FJ, Schlosser PM, and Janszen DB, Haber's rule: A special case in a family of curves relating concentration and duration of exposure to a fixed level of response for a given endpoint, *Toxicology* **149**:21–34 (2000).

131 Winks RG, The significance of response time in the detection and measurement of fumigant resistance in insects with special reference to phosphine, *Pestic Sci* **17**:165–174 (1986).

132 Price NR, The mode of action of fumigants, *J Stored Prod Res* **21**:157–164 (1985).

133 Winks RG and Waterford CJ, The relationship between concentration and time in the toxicity of phosphine to adults of a resistant strain of *Tribolium castaneum* (Herbst), *J Stored Prod Res* **22**:85–92 (1986).

134 Walse SS and Jimenez LR, Postharvest fumigation of fresh citrus with cylinderized phosphine to control bean thrips (Thysanoptera: Thripidae), *Horticulturae* **7**:134 (2021).

135 Nitschke KD, Albee RR, Mattsson JL, and Miller RR, Incapacitation and treatment of rats exposed to a lethal dose of sulfuryl fluoride, *Toxicol Sci* **7**:664–670 (1986).

136 Mendrala AL, Markham DA, and Eisenbrandt DL, Rapid uptake, metabolism, and elimination of inhaled sulfuryl fluoride fumigant by rats, *Toxicol Sci* **86**:239–247 (2005).

137 Meikle RW and Stewart D, Fumigant mode of action: Drywood termite metabolism of vikane fumigant as shown by labeled pool technique, *J Agric Food Chem* **11**:226–230 (1963).

138 Nicholls P, Formate as an inhibitor of cytochrome c oxidase, *Biochem Biophys Res Commun* **67**:610–616 (1975).

139 Siedow JN and Umbach AL, The mitochondrial cyanide-resistant oxidase: Structural conservation amid regulatory diversity, *Biochim Biophys Acta - Bioenerg* **1459**:432–439 (2000).

140 Saari S, Kemppainen E, Tuomela T, Oliveira MT, Dufour E, and Jacobs HT, Alternative oxidase confers nutritional limitation on Drosophila development, *J Exp Zool Part A Ecol Integr Physiol* **331**:341–356 (2019).

141 Ormsby M, Technical review - proposed treatments for BMSB (*Halyomorpha halys* (Stål); Pentatomidae) technical review – proposed treatments for BMSB, 1–38 (2018).

142 Schortemeyer M, Thomas K, Haack RA, Uzunovic A, Hoover K, Simpson JA, *et al.*, Appropriateness of probit-9 in the development of quarantine treatments for timber and timber commodities, *J Econ Entomol* **104**:717–731 (2011).

143 Haack RA, Uzunovic A, Hoover K, and Cook JA, Seeking alternatives to probit 9 when developing treatments for wood packaging materials under ISPM No. 15, *EPPO Bull* **41**:39–45 (2011).