Methods for management of *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae) in dry-cured ham facilities

by

Salehe Abbar

B.S., Shahid Bahonar University of Kerman, Iran, 2004
M.S., Ferdowsi University of Mashhad, Iran, 2008

AN ABSTRACT OF A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Entomology
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2016

Abstract

Dry-cured ham is protected from infestations of *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae) with the fumigant methyl bromide. Developing feasible alternatives to methyl bromide is necessary due to the phase out of methyl bromide. The effectiveness of food-safe compounds for preventing infestations of *T. putrescentiae* on dry-cured hams was evaluated by dipping ham pieces in solutions of various food additives. Propylene glycol (1, 2-propanediol), lard, ethoxyquin and butylated hydroxytoluene prevented or significantly reduced mite population growth. A combination of carrageenan + propylene glycol alginate + 40% propylene glycol was effective in reducing mite numbers on treated whole-aged hams compared with untreated hams.

Dose/response tests with twelve registered residual insecticides were conducted to assess contact toxicity to *T. putrescentiae*. Three of these insecticides were evaluated for persistence over a 2-month period on different surfaces. Commercial formulations of deltamethrin plus chlorpyrifos-methyl, chlorfenapyr, and malathion showed promising results for contact toxicity against *T. putriscentiae*. Chlorfenapyr applied to metal, concrete, and wood resulted in 100% mortality of treated ham mites for up to 8 weeks.

The effect of high and low temperatures on mortality of *T. putrescentiae* was studied in the laboratory. Groups of 10 eggs and groups of a mixture of 40 adults and nymphs were separately exposed to several high and low temperatures, ranging from +35 to 45°C and from −20 to +5°C, for several time periods. Eggs were found to be more tolerant to both high and low temperatures compared with adults and nymphs. Results showed that high temperatures from 40-45°C killed all *T. putrescentiae* eggs, adults, and nymphs within 4-1 d, while −10°C or lower killed all the same stages in less than 1 d.

Combinations of the fumigant sulfuryl fluoride (SF) applied under high temperatures ranging from 25-40ºC, were studied to determine the highest temperature, shortest exposure time, and the lowest value of a concentration-by-time product (CTP) of SF against *T. putrescentiae*. Results showed that complete control of all life stages of *T. putrescentiae* was achieved at 40ºC with SF. More than one day of exposure was required to kill adults and nymphs and eggs at 40ºC at a CTP close to the EPA labeled rate of 1500 $gh.m^{-3}$. Results indicated that adults and nymphs were more susceptible to SF compared to eggs. This study focused on investigating different control methods for *T. putrescentiae* in dry-cured ham facilities and most of examined techniques are preventive, although some of them can be applied as remedial methods after mite infestations are noticed.

Methods for management of *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae) in dry-cured ham facilities

by

Salehe Abbar

B.S., Shahid Bahonar University of Kerman, Iran, 2004
M.S., Ferdowsi University of Mashhad, Iran, 2008

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Entomology
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2016

Approved by:

Co-Major Professor
Thomas W. Phillips

Approved by:

Co-Major Professor
Robert Jeff Whitworth

Copyright

Abstract

Dry-cured ham is protected from infestations of *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae) with the fumigant methyl bromide. Developing feasible alternatives to methyl bromide is necessary due to the phase out of methyl bromide. The effectiveness of food-safe compounds for preventing infestations of *T. putrescentiae* on dry-cured hams was evaluated by dipping ham pieces in solutions of various food additives. Propylene glycol (1, 2-propanediol), lard, ethoxyquin and butylated hydroxytoluene prevented or significantly reduced mite population growth. A combination of carrageenan + propylene glycol alginate + 40% propylene glycol was effective in reducing mite numbers on treated whole-aged hams compared with untreated hams.

Dose/response tests with twelve registered residual insecticides were conducted to assess contact toxicity to *T. putrescentiae*. Three of these insecticides were evaluated for persistence over a 2-month period on different surfaces. Commercial formulations of deltamethrin plus chlorpyrifos-methyl, chlorfenapyr, and malathion showed promising results for contact toxicity against *T. putriscentiae*. Chlorfenapyr applied to metal, concrete, and wood resulted in 100% mortality of treated ham mites for up to 8 weeks.

The effect of high and low temperatures on mortality of *T. putrescentiae* was studied in the laboratory. Groups of 10 eggs and groups of a mixture of 40 adults and nymphs were separately exposed to several high and low temperatures, ranging from +35 to 45°C and from −20 to +5°C, for several time periods. Eggs were found to be more tolerant to both high and low temperatures compared with adults and nymphs. Results showed that high temperatures from 40-45°C killed all *T. putrescentiae* eggs, adults, and nymphs within 4-1 d, while −10°C or lower killed all the same stages in less than 1 d.

Combinations of the fumigant sulfuryl fluoride (SF) applied under high temperatures ranging from 25-40ºC, were studied to determine the highest temperature, shortest exposure time, and the lowest value of a concentration-by-time product (CTP) of SF against *T. putrescentiae*. Results showed that complete control of all life stages of *T. putrescentiae* was achieved at 40ºC with SF. More than one day of exposure was required to kill adults and nymphs and eggs at 40ºC at a CTP close to the EPA labeled rate of 1500 $gh.m^{-3}$. Results indicated that adults and nymphs were more susceptible to SF compared to eggs. This study focused on investigating different control methods for *T. putrescentiae* in dry-cured ham facilities and most of examined techniques are preventive, although some of them can be applied as remedial methods after mite infestations are noticed.

Table of Contents

List of Tables

Acknowledgements

First and foremost, I would like to express my profound gratitude to God, the almighty for providing me this opportunity and granting me the capability to proceed successfully.

I would like to express my sincere appreciation and gratitude to my major advisor Prof. Thomas W. Phillips for the continuous support of my Ph.D. study and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this dissertation. I could not have imagined having a better advisor and mentor for my Ph.D. study. My sincere thanks also goes to Dr. Robert Jeff Whitworth, who provided me financial support and an opportunity to join K-State department of entomology. He let me join his field research group and taught me about field crop insect pests. Without his generous support it would not be possible to accomplish my Ph.D. I would like to express my genuine appreciation to Prof. M. Wes Schilling who unwaveringly supported me to conduct this research and to publish related articles. I would like to thank the rest of my Ph.D. committee: Prof. David Hagstrum, and Prof. Bhadriraju Subramanyam, for their insightful comments and encouragement.

I thank my fellow lab mates: Michael Aikins, Barbara Amoah, Edwin Afful, Stephen Losey, Aaron Cato, Dr. Ozgür Saglam, Dr. Mukti Ghimire, and Dr. Zhaorigetu Chen for the inspiring discussions, and for all the fun we have had in the last four years. I also thank all members of the Department of Entomology. I am grateful for your contributions to my experience here. I am grateful for the financial support from the US Department of Agriculture Methyl Bromide Transition Program and K-State research and extension program.

Last but not the least, I would like to thank my family: my parents, M. Abbar and Z. Raoufifard, and my brothers, Sajad and Sobhan, and their wives, Maria and Nafiseh for supporting me spiritually throughout doing this research and my life in general.

Dedication

I humbly dedicate this work to

My loving parents, whose affection, encouragement

and prayers of day and night made me successful,

along with all hard working and respected teachers.

Chapter 1 - Introduction and Objectives

Dry-cured hams and related pests

The history of preparing dry-cured ham can be traced back to ancient times when dry curing initially was used as a preservation process. Today, salting is not needed as a preservative method for meats due to refrigeration technology, and incorporation of additives like nitrates and ascorbic acid to preserve and produce a tasty meat product (Toldrá et al. 1997). American dry-cured ham, also known as country ham, is a treasured tradition, produced from a hog`s hind leg and cured with dry salt through different procedures depending on the region (Marriott and Ockerman 2004). By definition, true American dry-cured ham is cured with a dry salt cure, loses at least 18% of its original weight in curing and aging process from dehydration, and contains a minimum of 4% salt (USDA 1999). This special type of ham is therefore cured with salts, sometimes smoked, and then aged for several months (Marriott and Ockerman 2004). Based on traditional procedures, preparing dry-cured ham involves several steps: curing, salt equalization, and aging (Zhao et al. 2016). The typical curing ingredients for American dry-cured hams include salt, sodium nitrate, sugar, and spices and each has a pivotal role the in curing process (Marriott and Schilling 2004). Salt inactivates spoilage microorganism proliferation through reduction of water activity and elevates the solubility of myofibrillar proteins. Nitrate breaks down to nitric acid and changes the flavor and color of the ham to pink (Toldrá et al. 1997, Armenteros et al. 2012). During the curing step, hams are placed fat side down in one layer and completely surrounded by salt. Hams are typically kept for 6 weeks at temperatures between 2 and 4°C, while treated with curing mixture and length of this stage can vary based on the weight of the fresh ham, and the processor's preference for meeting USDA requirements (Toldrá et al. 1997, USDA 9 CFR 318.10 2011). Ingredients are dissolved by the ham`s natural moisture and diffuse slowly into the ham. The curing mixture is removed from surfaces of hams in the next

stage, usually by washing with water, and hams are then prepared for smoking when it favors the processors. Hams are stored at 10-13°C for about 2 weeks after curing stages for cure equalization. The hams shrink approximately 8-10% during curing treatment and equalization (Marriott and Schilling 2004, Graham et al. 2012). The relative humidity is less than 75% during this process to inhibit microbial growth and the production of unpleasant flavors (Marriott and Ockerman 2004). The last and more complex stage is the aging or ripening period. Hams are placed in ventilated rooms and kept under time-temperature-relative humidity cycles (Graham et al. 2012). Temperatures vary between 16 and 25°C during the aging for European dry-cured ham with relative humidity varying between 65 and 80%, while aging temperatures may go as high as 28°C or more in the United States (Toldrá 2010, Rentfrow et al. 2012). More than half of country ham producers in the US age hams for 3-6 months. However many companies continue aging hams between 6 months to 2 years to intensify the ham flavor (Rentfrow et al. 2012). During the aging stage, hams lose 8-12% of their original weight (Graham et al. 1998,). The loss of moisture gives the ham a deeper color, firm texture, and desirable taste depending on the length of aging (Toldrá et al. 1997). The final product is a shelf-stable, dry-cured ham with unique flavor (Flores and Toldrá 1993). Country hams have considerable economic importance. In 2005, the National Country Ham Association (NCHA) members, including 20 companies, produced 3.4 million hams with the retail value of more than 340 million dollars. The total number of dry-cured hams produced in US in 2005, by both NCHA members and non-members, was estimated to exceed 6.5 million (Ramos 2006).

Pest infestation of dry-cured hams

Dry-cured hams contain a high level of protein and moisture, offering a good quality food source to different pests such as the ham mites *Tyrophagus putrescentiae* (Schrank) (Sarcoptiphormes: Acaridae), larder beetles, *Dermestes lardarius* L. (Coleoptera: Dermestidae**)**, red-legged ham beetles, *Necrobia rufipes* (F.) (Coleoptera: Cleridae), and cheese skippers, *Piophila casei* (L.) (Diptera: Piophilidae) (Graham et al. 2012). Ham mite was first described by Schrank in 1781(Klimov and Oconnor 2010). It is an astigmatid mite in the family Acaridae. Astigmated mites lack stigmata or external breathing pores (Olsen 1995). Schrank proposed the name *Acarus putrescentiae* for this species collected from the 'Austrian Empire' in garden soil, flower pots, and rotting leaves. There are other synonyms for *Tyrophagus putrescentiae* such as *Tyroglyphus lintneri* Osborn (1893) and *Tyroglyphus castellanii* Hirst (1912), but an extensive survey indicated that the name *Tyrophagus putrescentiae* has been used in a majority of studies published during the past 20 years (Klimov and Oconnor 2010, Farrell and Nayak 2012). This mite is known under several common names such as mold mite, copra mite, cheese mite, cereal mite, forage mite, mill mite, and ham mite (Rentfrow et al. 2008, Farrell and Nayak 2012). Description and keys to identify storage mites have been provided by Robertson (1959) and Kucerova and Stejskal (2009).

Adults are small, less than 350 μm long, pearly white, and slow moving. Their body's cuticle is colorless (Edde et al. 2012). Principal body hairs, including 4-7 pairs at the posterior end of body, are long, flexible, either smooth or with minute pectinations. This mite has slender legs and a pair of opistonotal glands, located dorso-laterally and filled with a colorless liquid that turns dark after death and appears as a pair of large brown spots behind the posterior legs (Farrell and Nayak 2012). The eggs of *T. putrescentiae* are about 122.2 μm in length and 69.8 μm in

width (Kucerova and Stejskal 2009). Developmental stages of *T. putrescentiae* consist of egg, larva, resting larva, protonymph, resting protonymph, deutonymph, resting deutonymph, and adult (Hilsenhoff and Dicke 1963). The age structure of *T. putrescentiae* is affected by environmental factors. Under ideal conditions of food, and temperature and relative humidity, eggs make up about 50% of the population, and other mobile (nymphs/adults) and immobile resting life stages constitute the rest of the population (Boczek 1991). Egg and larval development complete at optimum temperature of 32.2°C and 98% RH, in about 80 and 139 hours, respectively (Barker 1967). According to Bahrami et al. (2007), typical development times of egg, larva, and the two nymphal stages are 2, 3 and 5 days, respectively at a temperature of 25°C and 70% RH. There are different reports on longevity of adult mites depending on temperature, relative humidity, and food source. Lifespan of females and males reared on wheat germ at 25°C and 85% RH was 66 and 59 days, respectively. Longevity of adult mites at temperature of 9.3°C was 115 d and at 31°C was 42.8 d (Boczek, 1991). Hilsenhoff and Dicke (1963) showed that *T. putrescentiae* completed one life cycle on cheese in 38 d at 13ºC and 100% RH. Lifespan increased to 80 days when relative humidity changed from 75 to 85% at 26°C and at a relative humidity higher than 85%, lifespan decreased. In terms of food impact on longevity, it was up to 120 d for mites reared on wheat germ, 80 d on pumpkin seeds, 75 d on powdered milk and 25 d on rolled oats (Boczek, 1991). Population of *T. putrescentiae* is female dominated. Adults mate soon after eclosion and females lay their first batch of eggs within 24 h after mating, but starved, mated females are not able to lay eggs. About 70% of eggs are produced during the first three weeks of life (Boczek, 1991). A female reared on wheat germ or yeast can produce 500 eggs. Number of eggs produced per female vary based on the food substrate. On rolled oats, dried plums, cotton seeds, dried milk, buck wheat, and pollen the

number of eggs per female were 8, 12, 84, 85, 150, and 600, respectively (Rivard 1961, Boczek 1991, Chmielewski 1999, Bahrami et al. 2007). The capacity of egg laying for each female can be 60 egg/day, but the average is 4 per day (Boczek, 1991).

T. putrescentiae is a cosmopolitan species that can be found in various habitats including stored products like grain, whole wheat flour, wheat germ, soy flour, peanuts, medicinal herbs, cheese, nuts, copra (dried coconut), dried eggs, cotton seed, rapeseed, sunflower seed, dried banana, tobacco, bacon, dried milk, mushroom, dry-cured ham, house dust, pollen, soil, litter and the nests of different animals (Farrell and Nayak 2012). It has also been found in greenhouses in flower heads of Gerbera (*Gerbera sp*. L., Asteraceae), Nerine (*Nerine sp*. Herb., Amaryllidaceae) and Kalanchoe (*Kalanchoe sp*. Adans., Crassulaceae) leaf tissue (Robertson 1946, Boczek 1991, Duek et al. 2001, Bahrami et al. 2007, Kheradmand et al. 2007). *T. putrescentiae* is a predator of some nematodes including *Aphelenchus avenae* Basin and *Meloidogyne javanica* (Treub) (Bahrami et al., 2007). This mite also is a potential vector of parasitic and saprophytic fungi, like *Aspergillus flavus* Link*, Neosartorya fumigata* O'Gorman, Fuller & Dyer*, Aspergillus niger* Van Tiegham*, Mucor racemosus* Bull and *Nectria haematococca* (Fr.) and by transporting these pathogens may cause significant economic damage in mushroom beds (Kheradmand et al., 2007). *T. putrescentiae* has been indicated as an egg predator of southern corn rootworm, *Diabrotica undecimpunctata* (L.) (Coleoptera: Chrysomelidae) (Brust and House 1988), under no-till condition. It can cause allergic reactions that induce asthma, perennial rhinitis, conjunctivitis, intestinal acariasis and dermal allergies to humans. When hay and other fodders are infested, these mites cause intestinal disorders in horses and other animals (Farrell and Nayak 2012). This mite dispersal to new places is through contaminated foodstuffs, plant and animal materials, or they move on the bodies of birds or

animals that have used mite-contaminated nests. Food processing machinery and facilities can provide refuges for mites after which they can infest the new products during manufacture (Farrell and Nayak 2012). Ham mite infestations in dry-cured ham facilities vary often closely related to the length of aging process due to the cured hams becoming more favorable to ham mites (Rentfrow et al. 2006, Toldrá et al. 1997). Mite infestations commonly happen on the surface of dry-cured hams and every so often mites may penetrate inside the hams and thus cause more severe economic losses (Zdárková 1991). Rentfrow et al. (2006) reported that facilities in which cured hams were aged more than five months and with less sanitation struggled more with ham infestations in comparison with ones that aged cured hams less than five month.

N. rufipes, red-legged ham beetles, is a cosmopolitan pest that infests various stored-products with high protein content like copra, cheese, dried fish, ham and cause substantial damage (Simmons and Ellington 1925). Adults are approximately 3.5 to 7 mm long with pale yellowish to reddish colored legs, and a dark blue to metallic green body color (Lambkin and Khatoon 1990, Odeyemi 1997). The life cycle ranges from 1 to 5 months or more based on the types of available food and temperature. The optimum development conditions for *N. rufipes* are 30-34°C and 50% or higher relative humidity (Haines and Rees 1989). Adult red-legged beetles may live for more than 14 months, and females can lay up to 28 eggs per day and 3000 eggs in one life cycle under warm (30 to 33°C) and humid conditions (Roesli and Subramanyam 2002).

Cheese skippers, *P. casei,* sometimes called ham skippers, are detritivores that are often found on overripe/moldy cheese and salted meats such as dry-cured ham, bacon and beef (Lewis and Kaufman 2010). Adults are small metallic-colored flies and approximately half the size of the common house fly. Females lay 140-500 eggs on meat and cheese in one life cycle. A complete life cycle length is from 12 days to a month and adults lives for three to seven days

(Lewis and Kaufman 2010). Burrowing larva is the damaging stage, penetrating and feeding deeply into the materials (White et al. 2006).

Larder beetle, *D. lardarius,* is a cosmopolitan species and a common pest of cured meats, cheeses, dried fish, dried museum specimens and pet food (Haines and Rees 1989). Adults are 7–9 mm long, dark brown with a yellowish band across the front portion of the forewings. Females lay more than 100 eggs through the summer months and life cycle takes less than 2 months to be completed under ideal conditions (Jacobs 2013).

Fumigants for controlling ham mite infestation

By definition a fumigant is a toxic chemical gas with the capacity for penetration into porous objects or throughout enclosed areas, under a range of temperatures and pressures, for eradication of all developmental stages of a pest organism (Thoms and Phillips 2004). Phillips et al. (2012) stated that major factors affecting proper fumigant application are made up of target pest, type of commodity fumigated, most tolerant life stage to that particular fumigant, fumigant concentration, length of exposure period, and the temperature of treated environment.

Methyl bromide

Methyl bromide (CH_3Br) is an odorless, colorless and broad spectrum fumigant that can eliminate insects, mites, rodents, micro flora and nematodes and it has been used for more than 40 years commercially (Fields and White 2002). Methyl bromide can diffuse evenly throughout the fumigated space and commodity to achieve a constant exposure for pest disinfestation. Methyl bromide leaves little to no residues on commodities and is known as nonflammable and noncorrosive (Bond 1984). Dry-cured hams in the United States are considered adulterated if there is any mite infestation during the aging period, and it is mandatory for processors to remove mites (USDA 9 CFR 301 2011, USDA 9 CFR 416 2011). Methyl bromide has been the only known fumigant capable of ham mite controlling (Marriott and Schilling 2004). Thirty four dry-cured ham processing facilities were surveyed in 2006 in North Carolina, Virginia, Kentucky, Missouri, Tennessee and Georgia of which 20-25 were known to be major ham producers (Rentfrow et al. 2008). The reports showed 22 of these surveyed facilities used methyl bromide for mite control and almost half of these facilities use methyl bromide to control red-legged ham beetles. The total amount of methyl bromide applied by these 34 facilities was approximately less than 10,000 kg per year. Methyl bromide was used 1 to 5 times a year to

control *T. putrescentiae* and *N. rufipes*. Furthermore, survey results revealed that less than 1000 kg/year of methyl bromide is used by other dry-cured ham facilities in the United States which were not included in the survey (Schilling et al. 2010). Methyl bromide is classified as a Class 1 ozone depleting substance and its production and use have to be regulated under the United States Clean Air Act (US EPA 2016). According to the Montreal Protocol, which is an international treaty developed to protect the earth from detrimental effects of ozone depleting substances and signed by 196 countries, methyl bromide was to be phased out of according to time line for developed and developing countries (US EPA 2010). Therefore, there is a significant need to find safe, feasible, economic, and effective alternative compounds and methods in place of methyl bromide. According to the Montreal Protocol and the United States Clean Air Act, only critical use exemptions (CUE) for methyl bromide were nominated and approved to provide time for industries to evaluate methyl bromide alternatives and provide enough time to transition from using methyl bromide to using alternatives. One of the CUE, issued by EPA involves post-harvest uses including dry-cured pork products (US EPA 2012). Consequently, methyl bromide has been used in the ham-processing industry under a CUE since there were no suitable alternatives to control insects and mites associated with dry-cured hams (US EPA 2012). The nomination amount of CUE of methyl bromide for post-harvest dry-cured ham in 2016 was 3,240 kg/year, which is comparatively less than the nominated amount of methyl bromide (4,465 kg) in 2010 (Porter et al. 2014).

Potential viable alternatives to methyl bromide

Some potential alternatives to methyl bromide have been investigated and there are a few reports on their effectiveness or feasibility (Zhao et al. 2016). Application of lard on dry-cured ham (García 2004) and contact insecticides in aging rooms of ham facilities are known preventive techniques against *T. putrescentiae* infestation (Graham et al. 2012). The majority of research on methyl bromide alternatives in dry-cured ham plants was initiated after 2007. In these studies, the efficacy of sulfuryl fluoride, phosphine, carbon dioxide, and ozone to control *T. putrescentiae* and *N. rufipes* was investigated under laboratory conditions.

Sulfuryl fluoride

Sulfuryl fluoride (SF) is an odorless and colorless fumigant and its most common use is to control household infestations and structural pests such as dry wood termites (Kalotermitidae), bed bugs, *Cimex lectularius* Latreille, and cockroaches (Cochran and DiPaolo 2006). ProFume®, which is a trade name for one of the SF products, was developed as a replacement for methyl bromide for post-harvest settings (Phillips et al. 2012). SF was registered for use in dry cure ham facilities in 2005 (EPA 2005). SF is a broad spectrum fumigant with great efficacy against a broad range of insect pests and has good diffusion throughout the applied area (Baltaci et al. 2009). Fluoride anion is a product of SF degradation and is a post-fumigation residue in food that is regulated. Penetration capacity of SF is 10 times greater than methyl bromide and it is a non-ozone depleting fumigant (Rajendran 2001). SF is a promising fumigant against stored-product insects (Zettler et al. 2000). Phillips et al. (2008) reported that SF was effective for controlling all life stages of *N. rufipes* adults at concentrations below the label rate. They also showed SF killed mobile life stages of *T. putrescentiae* at 23ºC, but some eggs survived concentrations as high as three times the SF label rate. Egg tolerance to SF can be due to the impermeability of egg shell

that causes limitation of SF diffusion into the egg (Outram 1970). Thus, efficacy of SF maybe limited if eggs survival is high.

Phosphine

Phosphine (PH_3) is colorless and odorless gas, which has been used worldwide as an effective fumigant to protect stored products and disinfest storage facilities. The most important advantages of phosphine include its ease of application, low cost, and potency. The maximum allowable phosphine residues for stored-products is concentration of 0.01 ppm (USDA 40 CFR 180.225, 1999). Phosphine does not deplete the ozone layer and decomposes to safe compounds after diffusion into the environment (Chaudhry 1997, Zuryn et al. 2008). Phosphine is labeled for application to several stored products like cereals, nuts, dried fruits, pulses, oilseeds and dried animal products. It is commonly produced from formulations of aluminum or magnesium phosphide tablets, pellets, sachets, ropes, and blankets that react with water vapor to release phosphine. It is most effective at higher temperatures and at longer exposure times (Hole et al. 1976, Longobardi et al. 2008). Since phosphine molecular weight is similar to gases in ambient air, it can distribute or penetrate well and rapidly through targeted substrates and storage facilities.

In a laboratory investigation, *T. putrescentiae* was completely controlled by phosphine under controlled conditions (3.7 L gas-tight jars in controlled environment chambers). With a 48 h exposure of 1000 ppm of phosphine, 100% mortality of all stages of *T. putrescentiae* was achieved (Sekhon et al. 2010a). Phillips and Schilling (2013) showed that complete control of *T. putrescentiae* occurred in aging rooms fumigated with phosphine. Substantial corrosion to electrical wiring and equipment was observed three weeks after the fumigation in treated aging room. Phosphine fumigation in a simulated dry-cured ham aging room resulted in 99.8%

mortality of *T. putrescentiae* in two weeks after fumigation (Zhao at al. 2015). Thus, phosphine could be a potential alternative to methyl bromide for ham mite control, but it has some limitations including flammability or explosion hazards. Furthermore, phosphine is corrosive to certain metals like gold, silver and most importantly, copper, which makes another shortcoming for its application. All electrical appliances, wiring, lighting, electronic equipment with integrated circuits, computer chips, telephones and other similar devices can be damaged under phosphine fumigation at higher humidities (Phillips et al. 2012).

Ozone

Ozone is a well-known antimicrobial agent due to its potential capacity for oxidization. It is generally recognized as a "safe (GRAS) substance" by the United States Food and Drug Administration (FDA) in 1982 (Guzel-Seydim et al. 2004). Also, ozone has been approved for use as a disinfectant or sanitizer in foods for food processing in the United States (Guzel-Seydim et al. 2004). Ozone can be applied as a fumigant to protect food storage room and packaged commodities against insect pests and pathogens. Previous research showed that the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) adults were the most susceptible among six stored-product insect pests studied. Bonjour et al. (2008) showed 100% mortality after 2 days exposure to 50 ppm and 4 days exposure to 25 ppm, respectively. Complete mortality of all life stages of *T. putrescentiae* was achieved at an ozone concentration of higher than 175 ppm after 48 h of exposure (Hasan et al. 2016). Ozone leaves no residue because it has unstable structure and decays naturally into oxygen; however, it does not penetrate porous surfaces as would other fumigants, while ham mites penetrate into meat surfaces. In addition, ozone can potentially cause oxidation of food products which results in off odors and it is expensive to use (Sekhon et al.

2010b). Hence, ozone may not be a suitable potential alternative to methyl bromide for disinfesting hams directly, but it could have potential as a sanitation and a space disinfestation method.

Carbon Dioxide

Carbon dioxide (CO_2) could be used as a fumigant to control various insect pests and does not leave a residue (Hasan et al. 2016).The effectiveness of CO_2 has been studied on black field crickets (Stevenson and Hurst 1995), green peach aphids (Van Epenhuijsen et al. 2002) and two spotted spider mites (Mitcham et al. 1997). CO_2 fumigation causes 100 % mortality in onion thrips at a 30% or more concentration after at least 24 h (Page et al. 2002). Efficacy of CO_2 relies on keeping an elevated CO_2 atmosphere sustained until all insects die, so ensuring a leak-proof system is necessary. The length of exposure time depends on concentration of CO_2 and environment temperature (Page et al. 2002). Other experimental results have shown mortality of all life stages of ham mite at concentration of 60% CO_2 with144 hours of exposure (Hasan et al. 2016). Two high pressures of CO_2, 15 and 20 bars, caused greater than 99% mortality for mixed stages of *T. putrescentiae* on artificial standard diets after 15 and 30 min, respectively (Riudavets et al. 2010). Increasing the pressure of CO_2 would probably increase the efficacy of the treatment and on the other hand the cost of treatment would increase which is counted as a drawback for this method (Riudavets et al. 2010). CO_2 application drawbacks consist of difficulties related to required long exposure time which interrupt the production process and is a safety hazard for people working close to ham aging rooms during fumigation due to high concentration of CO_2. Highly airtight structures are necessary to reach a high level of fumigant concentration and pest mortality. In addition, CO_2 is expensive and large quantities of a greenhouse gas must be

released to atmosphere which has its own environmental drawback (Phillips et al. 2012, Hasan et al. 2016).

Management of dry-cured ham insect/mite pests

Up to now, there are no practical alternatives to methyl bromide in dry-cured ham facilities. Therefore, the Clean Air Act and the Montreal Protocol permitted methyl bromide usage under critical use exemptions. However, based on EPA's annual inventory report in 2014, fumigation in dry-cured ham facilities would be dependent on available pre-phase-out stocks of methyl bromide, which are about 158 MT, and other alternatives must be practiced after the exhaustion of these supplies in the US (US EPA 2017). It is therefore important to develop effective and feasible alternatives to methyl bromide to maintain pest-free dry-cured ham facilities. Likewise, developing an Integrated Pest Management (IPM) program to control pests associated in these facilities is critical, as there is currently no comprehensive and practical IPM program specifically for ham mites in context of the dry-cured ham industry. Separate management possibilities have been studied and suggested for controlling *T. putrescentiae* previously (García 2004, Mueller et al. 2006, Hubert et al. 2007, Phillips et al. 2008, Sekhon et al. 2010a, Freitag and Kells 2013, Stara et al. 2014). IPM of *T. putrescentiae* has been neglected mainly due to the reliance on methyl bromide prior to its phase out. Two restricted IPM programs for *T. putrescentiae* have been proposed and studied by Rentfrow et al. (2006) and Nayak (2006). Sanitation practices in ham processing and aging rooms, and applying pesticides are major components of these programs. However, implementation of these tools might not easily provide a mite-free dry-cured ham facility as it has not been demonstrated in practice. Hence, improving and combining the current possible materials and techniques to control ham mite in dry-cured ham facilities and putting forward an environment- and customer-friendly IPM programs as alternative to methyl bromide are yet to realized.

This research was focused on investigating different control methods for *T. putrescentiae* to decrease populations of mites and to limit the occurrence of mites on dry-cured hams and surfaces in processing and aging rooms.

Objectives

1. Investigate the effects of food-safe compounds for their effects on *T. putrescentiae* population growth
2. Investigate the potential for edible gel coatings with food-safe additives on whole hams to protect against mite infestation
3. Evaluate the activities of registered pesticides from horticulture, field crops, and stored-products for their potential to control *T. puterescentiae*
4. Assess the residual activity of the most active pesticides on mortality rates of mites exposed to three different surfaces treated with these pesticides and aged up to 8 weeks
5. Determine the shortest effective exposure time over a range of low and high temperatures to control *T. putrescentiae* infestations
6. Determine the lowest necessary SF concentration, shortest required exposure time and the most reasonable high temperature for use in dry cured ham plants to control *T. puterescentiae*

References

Armenteros, M., M.-C. Aristoy, F. Toldra, and F. Toldr. **2012**. Evolution of nitrate and nitrite during the processing of dry-cured ham with partial replacement of NaCl by other chloride salts. Meat Sci. 91: 378–381.

Bahrami, F., K. Kamali, and Y. Fathipour. **2007**. Life history and population growth parameters of *Tyrophagus putrescentiae* (Acari: Acaridae) on *Fusarium graminearum* in laboratory conditions. J. Entomol. Soc. Iran. 26: 7–18.

Baltaci, D., D. Klementz, B. Gerowitt, M. J. Drinkall, and C. Reichmuth. **2009**. Lethal effects of sulfuryl fluoride on eggs of different ages and other life stages of the warehouse moth *Ephestia elutella* (Hübner). J. Stored Prod. Res. 45: 19–23.

Barker, P. S. 1967. The effects of high humidity and different temperatures on the biology of *Tyrophagus putrescentiae* (Schrank) (Acarina: Tyrogliphidae). Can. J. Zool. 45: 91–96.

Boczek, J. 1991. Mite pests in stored food. Ecol. Manag. food Ind. Pests.arlingt. FDA Tech. Bull. 4: 57–79.

Bond, E. J. 1984. Manual of fumigation for insect control. Rome: Food and Agriculture Organization.

Bonjour, E. L., C. L. Jones, R. T. Noyes, J. A. Hardin, R. L. Beeby, D. A. Eltiste, and S. Decker. **2008**. Efficacy of ozone against insect pests in wheat stored in steel grain bins, *In* Proc. 8th Int. Conf. Control. Atmos. Fumigation Stored Prod. P. pp. 522–529.

Brust, G. E., and G. J. House. **1988**. A study of *Tyrophagus putrescentiae* (Acari: Acaridae) as a facultative predator of southern corn rootworm eggs. Exp. Appl. Acarol. 4: 335–344.

Chaudhry, M. Q. 1997. Review A review of the mechanisms involved in the action of phosphine as an insecticide and phosphine resistance in stored-product insects. Pestic.

Sci. 49: 213–228.

Chmielewski, W. 1999. Acceptance of buckwheat grain as a food by *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae). 97: 95–97.

Cochran, R., and D. DiPaolo. 2006. Exposure assessment document for pesticide products containing sulfuryl fluoride. July Sacramento, CA. pp. 18-32.

Duek, L., G. Kaufman, E. Palevsky, and I. Berdicevsky. **2001**. Mites in fungal cultures. Mycoses. 44: 390–394.

Edde, P. A., M. Eaton, S. A. Kells, and T. W. Phillips. **2012**. Biology, Behavior, and Ecology of Pests in Other Durable Commodities, In Hagstrum et al (eds), Stored-product protection. S156. Kansas State University Research and Extension publication, Manhattan, KS, USA. pp. 45–62.

Farrell, J., and M. K. Nayak. **2012**. Diagnostic methods for mould mite Tyrophagus putrescentiae. http://pbt.padil.gov.au/pbt/index.php?q=node/46&pbtID=184

Fields, P. G., and N. D. G. White. **2002**. Alternatives to methyl bromide treatments for stored-product and quarantine insects. Annu. Rev. Entomol. 47: 331–359.

Flores, J., and F. Toldrá. **1993**. Curing: Processes and applications. Encycl. food Sci. food Technol. Nutr. 1277–1282.

Freitag, J. A., and S. A. Kells. 2013. Efficacy and application considerations of selected residual acaricides against the mold mite *Tyrophagus putrescentiae* (Acari: Acaridae) in simulated retail habitats. J Econ Entomol 106:1920–1926.

García, N. 2004. Efforts to control mites on Iberian ham by physical methods. Exp. Appl. Acarol. 32: 41–50.

Graham, P. P., R. F. Kelly, and N. G. Marriott. **1998**. Dry-curing Virginia style ham. Virginia

Polytechnic Institute and State University.

Graham, P. P., Marriott, and R. F. Kelly. **2012**. Dry-curing Virginia style ham. Virginia Polytech. Inst. State Univ.

Guzel-Seydim, Z. B., A. K. Greene, and A. C. Seydim. **2004**. Use of ozone in the food industry. LWT - Food Sci. Technol. 37: 453–460.

Haines, C. P., and D. P. Rees. **1989**. A field guide to the types of insects and mites infesting cured fish. Food & Agriculture Org.

Hasan, M.M., M. J. Aikins, W. Schilling, and T. W. Phillips. 2016. Efficacy of controlled atmosphere treatments to manage arthropod pests of dry-cured hams. Insects 7. Doi: 10.3390/insects7030044.

Hilsenhoff, W. L., and R. J. Dicke. **1963**. Effects of temperature and humidity on cheese mites. With review of literature. Mark. Res. Report.United States Dep. Agric. 599: 1–46.

Hole, B. D., C. H. Bell, K. A. Mills, and G. Goodship. **1976**. The toxicity of phosphine to all developmental stages of thirteen species of stored product beetles. J. Stored Prod. Res. 12: 235–244.

Hubert, J., V. Stejskal, Z. Munzbergova, J. Hajslova, and F. H. Arthur. 2007. Suppressive potential of bean (*Phaseolus vulgaris*) flour against five species of stored-product mites (Acari: Acarididae). J Econ Entomol 100:586–590.

Jacobs, S. B. 2013. larder beetle fact sheet. College of Agriculture Science. Cooperative extension. Entomol. notes. Department of Entomology. Pennsylvania State University.

Kheradmand, K., K. Kamali, Y. Fathipour, and E. M. Goltapeh. **2007**. Development, life table and thermal requirement of *Tyrophagus putrescentiae* (Astigmata: Acaridae) on mushrooms. J. Stored Prod. Res. 43: 276–281.

Klimov, P. B., and B. M. Oconnor. **2010**. *Acarus putrescentiae* Schrank, 1781 (currently *Tyrophagus putrescentiae*; Acariformes, Acaridae): proposed conservation of usage by designation of a replacement neotype. Bull. Zool. Nomencl. 67: 24–27.

Kucerova, Z., and V. Stejskal. **2009**. Morphological diagnosis of the eggs of stored-products mites. Exp. Appl. Acarol. 49: 173–183.

Lambkin, T. A., and N. Khatoon. **1990**. Culture methods for *Necrobia rufipes* (Degeer) and *Dermestes maculatus* Degeer (Coleoptera: Cleridae and Dermestidae). J. Stored Prod. Res. 26: 59–60.

Lewis, C., and P. E. Kaufman. **2010**. Cheese (or ham) skipper, *Piophila casei* (Linnaeus) (Insecta: Diptera: Piophilidae). university of Florida IFAS Extention.

Longobardi, F., M. Pascale, M. Silvestri, and A. Visconti. **2008**. Rapid method for determination of phosphine residues in wheat. Food Anal. Methods. 1: 220–225.

Marriott, N. G., and H. W. Ockerman. **2004**. The Ultimate guide to country ham: An American Delicacy. Brightside Press.

Marriott, N. G., and M. W. Schilling. **2004**. Dry cured pork research review white paper. National Country Ham Association, Inc. National Country Ham Association, *In* Annu. Meet. pp. 1–62.

Mitcham, E. J., S. Zhou, and V. Bikoba. **1997**. Controlled atmospheres for quarantine control of three pests of table grape. J. Econ. Entomol. 90: 1360–1370.

Mueller, D. K., P. J. Kelley, and A. R. VanRyckeghem. **2006**. Mold mites *Tyrophagus putrescentiae* (Shrank) in stored products, p. 1122. *In* 9th Int. Work. Conf. Stored Prod. Prot.

Nayak, M. K. 2006. Management of mould mite *Tyrophagus putrescentiae* (Schrank) (Acarina: Acaridae): a case study in stored animal feed. *Int Pest Control* 48:128-130.

Odeyemi, O. O. 1997. Interspecific competition between the beetles *Dermestes maculatus* DeGeer and *Necrobia rufipes* DeGeer in dried fish. Int. J. Trop. Insect Sci. 17: 213–220.

Olsen, A. 1995. Fundamentals of microanalytical entomology: a practical guide to detecting and identifying filth in foods. CRC Press.

Outram, L. 1970. Some effects of the fumigant sulfuryl fluoride on the gross metabolism of insect eggs. Fluoride. 3: 85–91.

Page, B. B. C., M. J. Bendall, A. Carpenter, and C. W. Van Epenhuijsen. **2002**. Carbon dioxide fumigation of *Thrips tabaci* in export onions. New Zeal. Plant Prot. 303–307.

Phillips, T. W., M. M. Hasan, M. J. Aikens, and M. W. Schilling. **2008**. Efficacy of sulfuryl fluoride to control ham mites and red-legged ham beetles. *In* Annu. Int. Res. Conf. Methyl Bromide Altern. Emiss. Reduction, Orlando, FL, Novemb.

Phillips, T. W., E. M. Thoms, J. DeMark, and S. Walse. **2012**. Fumigation, In Hagstrum et al (eds), Stored-product protection. S156. Kansas State University Research and Extension publication, Manhattan, KS, USA. pp. 157–179.

Phillips, T. W., and M. W. Schilling. 2013. Fumigation alternatives for the ham mite, *Tyrophagus putrescentiae*, p. 38. *In In* Annu. Int. Res. Conf. Methyl Bromide Altern. Emiss. Reduction, San Diego, CA, Novemb.

Porter, I., M. Pizano, and M. Besri. **2014**. Montreal protocol on substances that deplete the ozone layer. UNEP Technology and Economic Assessment Panel. UNEP, Ozone Secretariat, Nairobi.

Rajendran, S. 2001. Alternatives to methyl bromide as fumigants for stored food commodities.

Pestic. Outlook. 12: 249–253.

Ramos, A. M. C. do A. 2006. Molecular dissection of genomic regions influencing important economic traits in the pig. ProQuest.

Riudavets, J., C. Castane, O. Alomar, M. J. Pons, and R. Gabarra. 2010. The use of carbon dioxide at high pressure to control nine stored-product pests. J. Stored Prod. Res. 46: 228- 233.

Rentfrow, G., R. Chaplin, and S. P. Suman. **2012**. Technology of dry-cured ham production: Science enhancing art. Anim. Front. 2: 26–31.

Rentfrow, G., D. J. Hanson, M. W. Schilling, and W. B. Mikel. **2008**. The use of methyl bromide to control insects in country hams in the Southeastern United States. Ext. Publ. Kentucky Extension/National Ctry. Ham Assoc. ASC-171. 1–2.

Rentfrow, G., D. J. Hanson, M. W. Schilling, and W. B. Mikel. 2006. Methyl bromide use to combat mite infestation in dry-cured ham during production, In Proceedings, 2006 Annual International Research Conference on Methyl Bromide Alternatives and Emission Reduction, 5-9 November, Orlando, FL. pp. 108-1 to 108-2.

Rivard, I. 1961. Influence of temperature and humidity on longevity, fecundity, and rate of increase of the mite *Tyrophagus putrescentiae* (Schrank)(Acarina: Acaridae) reared on mold cultures. Can. J. Zool. 39: 869–876.

Robertson, P. L. 1946. Tyroglyphid mites in stored products in New Zealand. Department of Scientific and Industrial Research.

Robertson, P. L. 1959. A revision of the genus Tyrophagus, with a discussion on its Taxonomic position in the Acarina. Aust. J. Zool. 7: 146–182.

Roesli, R., and B. Subramanyam. **2002**. RLHB invade pet stores-pet store inventory was

overtaken by an unwelcome critter--The red-legged ham beetle. Pest Control. 70: 30–33.

Schilling, M. W., T. W. Phillips, J. M. Aikins, M. M. Hasan, R. K. Sekhon, and W. B. Mikel. **2010**. Research update: Evaluating potential methyl bromide alternatives for their efficacy against pest infestations common to dry cured ham. *In* Natl. Ctry. Ham Assoc. Annu. Meet. April 16th–17th, Asheville, NC.

Sekhon, R. K., M. W. Schilling, T. W. Phillips, M. J. Aikins, M. M. Hasan, A. Corzo, and W. B. Mikel. **2010a**. Effects of phosphine and methyl bromide fumigation on the volatile flavor profile and sensory quality of dry cured ham. Meat Sci. 86: 411–417.

Sekhon, R. K., M. W. Schilling, T. W. Phillips, R. M. J. Aikins, M. M. Hasan, R. Nannapaneni, and W. B. Mikel. **2010b**. Effects of carbon dioxide and ozone treatments on the volatile composition and sensory quality of dry-cured ham. J. Food Sci. 75: 452–458.

Simmons, P., and G. W. Ellington. **1925**. The ham beetle, *Necrobia rufipes* De Geer. Govern. Print. Office.

Stevenson, D. G., and P. L. Hurst. **1995**. Mortality of black field crickets (*Teleogryllus commodus*) in carbon dioxide atmospheres. New Zeal. J. Zool. 22: 381–385.

Thoms, E., and T. W. Phillips. **2004**. Fumigation. In Hand B. Pest Control. GIE Media, Inc.

Toldrá, F. 2010. Handbook of meat processing. In (Ed.), Handbook of meat processing. John Wiley & Sons, Chichester; UK:

Toldrá, F., M. Flores, and Y. Sanz. **1997**. Dry-cured ham flavour: enzymatic generation and process influence. Food Chem. 59: 523–530.

United States Environmental Protection Agency. **2005**. Sulfuryl fluoride: Pesticide Tolerance Federal Register Environmental Documents.

United States Environmental Protection Agency. **2010**. Methyl bromide critical use nominations for post-harvest use for commodities.

United States Environmental Protection Agency. **2012**. Methyl bromide critical use renomination for post harvest dry-cured pork products. United States Environmental Protection Agency.

United States Environmental Protection Agency. **2016**. Phaseout of class I ozone-depleting substances. Http://Www.Epa.Gov/.

United States Environmental Protection Agency. 2017. Methyl bromide critical use renomination for post-harvest – dry cured pork products. http://www3.epa.gov/ozone/mbr/2017CUNPork.pdf EPA, Washington, DC.

USDA. **1999**. Food ingredients and sources of radiation listed or approved for use in the production of meat and poultry products. Insp. Food Saf. Serv. 64: 72168–72195.

USDA 40 CFR 180.225. Phosphine; tolerances for residues.

USDA 9 CFR 301. **2011**. Prescribed treatment of pork and products containing pork to destroy Trichinae.

USDA 9 CFR 318.10. **2011**. Prescribed treatment of pork and products containing pork to destroy Trichinae.

USDA 9 CFR 416. **2011**. Prescribed treatment of pork and products containing pork to destroy Trichinae.

Van Epenhuijsen, C. W., A. Carpenter, and R. Butler. **2002**. Controlled atmospheres for the post-harvest control of *Myzus persicae* (Sulzer)(Homoptera: Aphididae): effects of carbon dioxide concentration. J. Stored Prod. Res. 38: 281–291.

White, G. B., A. L. Rosales, E. S. Evans, and J. A. Hogsette. **2006**. Filth flies: significance,

surveillance and control in contingency operations. Technical Guide No. 30. Publ. Distrib. by armed forces pest Manag. board, Washingt.

Zettler, J. L., J. G. Leesch, and R. F. Gill. **2000**. Chemical treatment alternatives for postharvest pests, *In* 24th Annu. Meet. Work. Phytosanitary Altern. to Methyl Bromide. San Diego, CA. pp. 32–35.

Zhao, Y., S. Abbar, B. Amoah, T. W. Phillips, and M. W. Schilling. **2016**. Controlling pests in dry-cured ham: A review. Meat Sci. 111: 183–191.

Zhao, Y., S. Abbar, T. W. Phillips, and M. W. Schilling. 2015. Phosphine fumigation and residues in dry-cured ham in commercial applications. Meat Sci. 107: 57-63.

Zuryn, S., J. Kuang, and P. Ebert. **2008**. Mitochondrial modulation of phosphine toxicity and resistance in Caenorhabditis elegans. Toxicol. Sci. 102: 179–186.

Chapter 2 - Efficacy of selected food-safe compounds to prevent infestation of the ham mite, *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae), on southern dry-cured hams

Abstract

Tyrophagus putrescentiae (Schrank) (Sarcoptiformes: Acaridae), is a serious mite pest of dried meats and cheeses. Infestations of *T. putrescentiae* are controlled with the fumigant methyl bromide, which is an ozone-depleting substance and is currently being banned in most countries. Effective alternatives to methyl bromide are needed. The objective of this research was to use laboratory assays to investigate the effectiveness of food-safe compounds for preventing infestation of *T. putrescentiae* on dry-cured hams. Ham pieces dipped in solutions of either propylene glycol (1, 2-propanediol), lard, ethoxyquin or butylated hydroxytoluene prevented or significantly reduced mite population growth. Our results also indicated that a combination of carrageenan + propylene glycol alginate + 40% propylene glycol was effective in reducing mite numbers on whole aging hams compared with untreated whole hams. Several food-safe compounds can prevent infestation of *T. putrescentiae* on dry-cured hams and may represent alternatives for managing this pest.

Introduction

Tyrophagus putrescentiae (Schrank) (Sarcoptiformes: Acaridae), commonly known as the mold mite, ham mite or cheese mite, is a widely distributed and common synanthropic stored-product pest (Qu et al. 2015). *T. putrescentiae* infests a large number of grains and other foods with high fat and protein contents with moisture content between 15 and 40%, such as dried eggs, seasoned hams, sausages, cheese and different kinds of nut (Lee et al. 2006, Macchioni et al. 2002, Hughes 1976). *T. putrescentiae* is a key pest in mushroom cultivation and storage facilities in China, and in pet food in Australia and the United States (Mueller 2010, Nayak 2006). *T. putrescentiae* causes heavy infestations on traditional ham in Spain and reduces the quality of this valuable product (Qu et al. 2015, Sánchez-Ramos and Castañera 2005). This mite has also been reported in animal feed factories, in house dust and as a pest in biological laboratory environments (Stara et al. 2014, Nayak 2006). A *T. putrescentiae* female can lay up to 500 eggs on appropriate diet, and each egg can develop to the adult stage in 9–12 days under suitable temperature and relative humidity conditions (e.g. 25°C and >65% RH) (Boczek 1991). *T. putrescentiae* infestation is a serious problem to both European and American dry-cured ham producers (Rentfrow et al. 2008, Sánchez-Ramos and Castañera 2001).

Dry curing is used to preserve pork without refrigeration and results in a specialty meat product of high value (Marriott and Schilling 2004). American dry-cured ham or 'country ham' is produced in the southern United States by treating the hind leg of a butchered hog with dry salts, preservative salts and other seasonings rubbed on the outer surface. Curing is sometimes followed by smoking to add flavor, and then by aging for several months under ambient conditions (Flores et al. 1997). Dry-cured hams lose at least 18% of their original weight during aging and will have a minimum of 4% salt (Marriott and Ockerman 2004). The aging process

can vary between 3 and 24 months, depending on the desired flavor, and longer-aged products have a higher market value (Marriott and Ockerman 2004). Dry-cured hams and processing facilities have been historically fumigated with methyl bromide to control ham mite infestations (US EPA 2012). However, methyl bromide is classified as a significant ozone-depleting substance (US EPA 2015) and is being phased out for all pest control uses for all industries. The US regulatory agencies consider ham mite infestation actionable, potentially resulting in cessation or delayed processing, with large economic losses to producers. Thus, methyl bromide has been an extremely important pest management tool for the dry-cured ham industry (Marriott and Schilling 2004). It is therefore vital for the dry-cured ham industry to implement effective alternatives to methyl bromide that can manage or control ham mites.

The present study addresses the potential for using GRAS food ingredients (Generally Recognized As Safe by the US Food and Drug Administration) to mitigate pest populations of *T. putrescentiae* and to protect hams from mite infestations during the aging process. Milne (1963) was among the first to demonstrate the control of stored-product insect pests by using food ingredients. These researchers reported that sorbic acid and nicotine suppressed cigarette beetle, *Lasioderma serricorne* (F.), infestations. Common cooking oils have been used for centuries to control mite and insect pests in horticulture, and many of them are still used to control crop and ornamental plant arthropod pests (Hoy 2011). The mode of action is presumed to be a physical blockage of respiration, with death resulting from suffocation (Lewis 2014). Larding, using lard or other animal fats as meat coatings, was recorded in England to enhance the shelf life of meat products (Labuza and Contreras-Medellin 1981). In addition, dry-cured hams are coated with hot lard in Spain to control mite infestations (García 2004). Edible food coatings have been applied to slow down water loss and give a shiny appearance to fruits and vegetables,

and meat products have been coated with gelatin to sustain quality (Baldwin et al. 2011). Edible coatings are inhibitory to off-flavors resulting from oxidation, discoloration, shrinkage and microbial contamination (Ustunol et al. 2009).

Recent work has evaluated xanthan gum, agar, propylene glycol alginate (PGA) and carrageenan + PGA for their efficacy in suppressing *T. putrescentiae* populations by using water solutions of propylene glycol (PG) at 10–50% (by weight). Results indicated that a combination of xanthan gum + 20% or more PG and a combination of carrageenan + PGA + 10% or more PG were effective at inhibiting mite population growth (Zhao et al. 2016). The objective of the research reported here was to investigate the effects of GRAS compounds and other food-safe compounds for their effects on *T. putrescentiae* population growth. This work also studied the potential for edible gel coatings with food-safe additives on whole hams to protect against mite infestation.

Materials and Methods

Mite cultures and hams used

The *T. putrescentiae* were from our laboratory cultures that have been maintained in the Department of Entomology at Kansas State University for more than 4 years and have not been subjected to any pesticides. Mites were reared in glass jars (85 mm diameter, 160 mm height) containing mite diet and sealed with labeled filter paper (Whatman No. 1, 90 mm diameter; GE Healthcare UK Limited, Amersham, UK) in the metal lid ring. The rearing diet was composed of agar (ICN Biomedicals, Inc., Aurora, OH), yeast (MP Biomedicals, LLC, Santa Ana, CA), alphacel (ICN Biomedicals, Inc.), mixed vitamins (Vanderzant modification vitamin mixture for insect diet; MP Biomedicals, LLC) (5:5:5:5 g), dog food (160 g), glycerol (Fisher BioReagents, Fisher Scientific, Pittsburgh, PA), antifungal salt solution in ethanol (methyl-p-hydroxybenzoate, 15:85 g mL^{-1}) (ICN Biomedicals, Inc.) and water (25:25:475 mL), which were mixed and cooked for about half an hour and then added to the dog food in rearing jars. Mites were introduced to new cultures when the diets had been cooled to 25°C and stored in an incubator at 25°C and 70% RH in darkness. All experiments described below used meat from whole country hams that were 6–8 kg and had been aged for at least 3 months at the time of purchase (Harper's Country Hams, Clinton, KY).

Mite reproduction assay

GRAS and other food-safe compounds (Table 2-1) were evaluated in separate experiments using completely randomized designs in which individual cubes of dry-cured ham ($25 \times 25 \times 25$ mm^3) were coated or dipped with test compounds. Ham cubes were dipped in 500 mL of a given solution for 1 min and were then left on filter paper in a 14 cm glass petri dish (Pyrex®, Frankfurt, Germany) to dry for 1.5 h. Treated ham cubes were placed separately inside

small glass Mason jars (216 mL, 65 mm diameter, 55 mm height; Ball Corp., Broomfield, CO) into which 20 adult mites were introduced for infestation. Ham cubes were coated with pure lard using a small artist's paint brush with natural bristles of approximately 5 mm width by 7 mm length, and were then placed in the test jars immediately. Other compounds were tested at one or several concentrations applied in a solvent or undiluted. Water was the solvent used for most test compounds, with other solvents and their test compounds as follows: ethanol (98% purified) for butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT); acetone (98% purified) for ethoxyquin; acetic acid (8% purified) for natamycin; hexane (97% purified) for lard. There were five experimental units for each concentration of a given compound in an experiment. Jars were sealed with labeled filter paper (Whatman No. 1, 70 mm diameter; GE Healthcare UK Limited) under the jar ring after mite inoculation to prevent escape of mites from the assay jar. The control treatment included untreated ham cubes and/or ham cubes treated with a given solvent, depending on the type of chemicals tested on treatment cubes. The numbers of living adults, larvae and nymphs after 2 weeks of incubation at 25°C and 70% RH with a 16:8 h L:D photoperiod were counted for each jar using a binocular microscope.

Mite residency on coated ham

Seven different treatments, including gel treatments and uncoated controls, were prepared. For each treatment, four whole hams were randomly selected as experimental units. Two experimental units of each treatment were randomly selected to be placed on each of two separate metal racks. The seven treatments included control, xanthan gum only, carrageenan + PGA only, 20% PG + xanthan gum, 40% PG + xanthan gum, 20% PG + carrageenan + PGA and 40% PG + carrageenan + PGA. Gel solutions of 300 mL were prepared in separate jars for each experimental unit (ham), and hams were coated with 150–200 mL of each gel using a paint brush

(5 cm width). Each ham was painted with gel treatments 3 times, with a 15 min period between each. Large S-hooks were attached to each ham with a bolt driven into the ham bone, and they were hung on metal racks for 24 h at 25°C and ≤40% RH. They were then transferred to a simulated ham aging room for mite residency studies. This room was established to mimic a commercial ham aging room and was maintained at 25°C and 70% RH in total darkness.

The residency of mites on treated hams was recorded at 6 and 12 weeks after hams were placed in the aging room. Attempts to create a ham mite infestation by artificially introducing approximately 14 000 lab-reared mites weekly failed to generate a detectable infestation at 6 weeks. Thus, about 900 mites were then introduced directly onto the top of each ham with an open 1.5 mL plastic centrifuge tube cap, and the number of mites remaining on each ham at 6 h was determined. At 12 weeks after the start of aging we observed an established endemic mite infestation, presumably a result of earlier inoculations, and we therefore counted resident mites at this time with no need for inoculation. Mites were counted in six randomly selected sampling areas on each ham for each observation period. Each sampling area was a circle of 23.8 cm^2, three on each side (proximal versus distal to the body of the pig) for each ham. Preliminary studies determined that representative mite samples could be obtained from the central 80% surface area of each ham side (i.e. excluding the top and bottom ends). Mites from randomly selected circular areas on each side of a given ham were visually counted without disturbance, and the sum of the six samples was recorded as a representative number of mites residing on a given ham.

Data Analysis

For mite reproduction assays the treatment means within an experiment were separated on the basis of Fisher's protected least significant difference (LSD) when the *F*-test of the

ANOVA per treatment was significant at $P < 0.05$ (SAS Institute Inc. 2010). Residency of mites on whole hams with different coatings was subjected to ANOVA for each observation time, and mean observation times were separated by the honest significant difference Tukey–Kramer test when the *F*-test of the ANOVA per treatment was significant at $P < 0.05$ (SAS Institute Inc. 2010).

Results

Mite reproduction assays

Experiments with solutions of the following nine compounds had no significant effects on mite reproduction relative to controls: corn oil, olive oil, calcium citrate, potassium citrate, sodium citrate, potassium propionate, potassium sorbate, sodium carbonate and natamycin. The results relating to all other tested compounds that had significant effects on mite reproduction and population growth suppression are reported in Tables 2-2 to 5. Mite reproduction in the five experiments conducted with ham cubes dipped in aqueous solutions of a variety of free acids and salts was significantly lower in all treatments compared with the water controls (Table 2-2), with calcium sorbate eliciting a fourfold reduction in mites produced. Vegetable oils and non-vegetable oils caused a reduction in mite reproduction compared with untreated ham cubes (Table 2-3). Canola oil caused a fourfold decrease in reproduction. Undiluted lard prevented all reproduction, and a 50% lard solution caused a threefold reduction in mites. Mite populations from ham cubes dipped in short-chain alcohols all had substantial reductions compared with the water controls, with the greatest effect recorded from 1,2-propanediol, commonly referred to as propylene glycol (PG). Ham cubes dipped into pure PG and also in a 50% water solution prevented all mite reproduction (Table 2-4). Of the food preservatives tested, 10% solutions of ethoxyquin and BHT totally prevented mite reproduction on treated ham cubes (Table 2-5).

Experiments with formulations of PG and carrageenan showed that carrageenan alone had a small but significant effect on mite reproduction (Table 2-6). Pure PG or when mixed with 2% carrageenan prevented mite reproduction completely, but dilutions of 50% or less allowed substantial mite reproduction (Table 2-6).

Mite residency on coated ham

Mite counts on whole aged hams coated with various formulations of PG in food gels at 6 h after inoculation with approximately 900 mites were lowest on those coated with 20% or 40% PG in carrageenan + PGA (Table 2-7). Carrageenan + PGA without PG reduced the number of mites on treated hams; however, it was not significantly different from mite numbers on untreated hams (Table 2-7). Xanthan gum alone or in combination with PG did not lower the mite populations significantly (Table 2-7). Results of counting mites on the same coated hams 12 weeks after treatment also revealed lower numbers in residence on hams with the combination of 20% PG in carrageenan + PGA, xanthan gum and carrageenan + PGA, but mite numbers on all other coated hams were not significantly different from numbers on untreated control hams (Table 2-8). Coatings of carrageenan + PGA + 40% PG had significant effects on lowering resident mite numbers when compared with the controls (Table 2-8).

Discussion

The results of this study provide information regarding the relationship between the effects of various food-safe compounds on ham mite population growth. Results from population growth experiments demonstrated that several food-safe compounds, specifically PG, BHT, lard, ethoxyquin and 1,3-butanediol, can inhibit growth of *T. putrescentiae* populations under experimental laboratory conditions. BHT was the most effective synthetic additive against *T. putrescentiae*, with 100% inhibition of population growth at a concentration of 10% in the dip. PG also inhibited mite populations by more than 99% at concentrations of 50 and 100%. Of the naturally derived oils tested, undiluted lard was also an effective treatment, providing more than 99% inhibition. The 10% concentration dip of ethoxyquin inhibited the populations by 96.4%, whereas the 1% dip produced an inhibition of 69%. Mite populations were inhibited by 96.7% when dipped in pure 1,3-butanediol. Compounds that inhibited mite population growth by 50–90% included calcium sorbate, sodium sorbate, 2-propanol, propionate salts, iodate salts, BHA, soybean oil, canola oil and lard at 50%.

Our experiments on organic alcohols suggest that activity against *T. putrescentiae* could be dependent on molecular structure, such as the number of carbons and alcohols in the molecule. Pankiewicz-Nowicka *et al.* (1986) studied the feeding and orientation of *T. putrescentiae* on artificial diets containing common food additives and reported equivocal structure–activity relationships for monosaccharides and their alcohols. In our work here with 3-carbon and 4-carbon polyols, mite population growth was minimally reduced by dips in the monoalcohols, but was dramatically lowered by diols of both 3-carbon and 4-carbon molecules, except for 1,2-butanediol. PG (1,2-propanediol) had the best suppression effects, but 1,2-butenediol, although very similar to PG by having its alcohols on adjacent carbons, was not very

effective. Thus, conclusions about structure–activity effects with short-chain diols and *T. putrescentiae* biology are not clear cut and will require further study.

Food-safe compounds such as those studied here are commonly used in the food industry to protect meats from microbial infection and to preserve quality. Sodium propionate at 0.03% inhibited aerobic and gram-negative bacterium population growth for about 2 weeks in fresh sausage, while calcium and sodium propionate also showed preventive effects on Salmonella Newport population growth for about 1 month in cooked chicken (Boudreaux and Matrozza 1993). Sorbate salts (calcium, sodium and potassium) had a great impact as inhibitors of fungi, including mycotoxin producers, and certain bacteria (Branen et al. 2001). Potassium sorbate at concentrations of 2.5–5 g 100 ml^{-1} dipping solution caused extensive inhibition of *Listeria monocytogenes* Pirie population growth in cooked pork (Samelis 2005). In addition, the antifungal efficacy of potassium sorbate against *Botrytis cinerea* Pers was determined in another study (Junqueira-Gonçalves et al. 2013).

The control of stored-product insect pests with food additives was suggested by Milne (Milne 1963), who found suppression effects of sorbic acid and nicotine on populations of cigarette beetle, *L. serricorne*. Propionic acid at 2% (by weight), BHA at 2% (by weight) and BHT at either 0.5 or 2% (by weight) caused significant mortality in first-instar larvae of both *Trogoderma variabile* Ballion (Coleoptera: Dermestidae) and *Attagenus megatoma* (F.) (Coleoptera: Dermestidae) (Burkholder et al. 1973). Citric acid is a common food preservative, but our work showed a positive, rather than negative, effect on ham mite population growth. Early work showed that a grasshopper bait containing citric acid stimulated feeding in *Camnula* sp. (Thorsteinson 1960). One study found that low efficacy of citric acid

on Varoa mite mortality can be a result of its poorer dissociation constant in comparison with other organic acids such as oxalic acid (Milani 2001).

The efficacy of various types of salt and free acid incorporated into mite diet has been examined in the past for mortality or fecundity of stored-product mites as chemosterilants (Collins 2006). Potassium iodate at >0.25% (by weight) suppressed egg laying of *T. putrescentiae* and *Acarus siro* (L.) (Sarcoptiformes: Acaridae) (Ignatowicz 1982a, 1982b). Boric acid produced 100% mortality of *T. putrescentiae* at >0.5% by weight (Ignatowicz 1986). Caproic acid and propionic acid at 1 and 2% (by weight) prevented 100% egg laying of *T. putrescentiae* (Rodriguez 1972). Tricalcium phosphate at 31.5% (by weight) reduced the fecundity of *T. putrescentiae* by 84% (Boczek and Ignatowicz 1978). Potassium sorbate, benzoic acid and calcium propionate at 0.01–1% (by weight) inhibited the development of *T. putrescentiae* and *A. siro* (Boczek and Czajkowska 1968). Sodium propionate, 3,3′-thiodipropionic acid, malonic acid and cholesteryl chloride reduced development of *T. putrescentiae* through immature life stages (Rodrigues and Potts 1975, Boczek and Czajkowska 1968).

Although lard showed strong inhibitory effects on mite population growth in our experiments, further studies are required to find a suitable way for the application of lard. Dry-cured hams in Spain are apparently protected from mite infestation by coating with hot lard (Baldwin et al. 2011), but no details are given for its application. American dry-cured hams need to lose at least 18% of the original water weight during the aging process (Marriott and Ockerman 2004), and simultaneously the presence of oxygen is needed for proteolysis and lipolysis, resulting in the characteristic flavors of dry-cured hams (Marriott and Schilling 2004). Thus, water vapor permeability of the lard needs to be considered when choosing it as a suitable

coating. Therefore, it is important to develop an economical food additive coating for preventing mite infestations that allows both high oxygen effects and water vapor permeability.

PG possesses fungicidal and bactericidal efficacy and inhibits odor-causing bacteria, animal-pathogenic bacteria, mites, fleas, red lice, Shigella bacteria, *A. niger* fungus and some other pathogens (US EPA 2006). The efficacy of PG has not been assessed previously on population growth inhibition of *T. putrescentiae* in the context of dry-cured ham. Results here showed that coating ham pieces with a 50% water solution of PG can provide the same prevention effects for mite infestation as dipping ham pieces in undiluted PG. Dipping in water solutions of PG may not be practical for protecting whole hams during the aging process, but our work with polysaccharide coatings containing PG shows promise for developing a commercial product (Zhao et al. 2016). The GRAS status of PG as a food-safe additive should not limit the amounts used as direct coatings for hams, but meat quality effects and consumer acceptance of such hams will require further research. Coatings for dry-cured hams may effectively prevent infestation of *T. putrescentiae*, but the adoption of such coatings in practice will require, among other considerations, an economic cost-benefit analysis. The loss of methyl bromide as a fumigant for control of *T. putrescentiae* has motivated the development of integrated pest management (IPM) for this important pest. A critical component of any effective IPM programme is the prevention of pest infestation. Our work on food-safe compounds to treat whole hams could contribute to effective prevention practices against *T. putrescentiae* infestations.

References

Baldwin, E. A., R. Hagenmaier, and J. Bai. 2011. Edible coatings and films to improve food quality. CRC Press, Boca Raton, FL.

Boczek, J. 1991. Mite pests in stored food, *In* Ecology and management of food-industry pests, ed. Gorham JR. AOAC Press, Arlington, VA. pp. 57-79.

Boczek, J., and B. Czajkowska. 1968. The effect of antimicrobial agents and antibiotics on some stored products mites (Acaroidea). Roczniki Nauk Rolniczych 93:597-612.

Boczek, J., and S. Ignatowicz. 1978. Effect of tricalcium phosphate on *Tyrophagus putrescentiae* (Schr.) (Acari: Acaridae), *In* 2nd Intl. Working Conf. Stored-Prod. 10–16 September 1978, Entomol. Ibadan, Nigeria. pp. 320-327.

Boudreaux, D. P., and M. A. Matrozza. 1993. Composition for extending the shelf life of processed meats. US Patent 5 219 603.

Branen, A. L. 2002. Food additives, vol. 116. CRC Press, Boca Raton, FL.

Burkholder, W. E., G. M. Schwalbe, and Boush. 1973. Antimicrobial food additives and their effects on *Trogoderma variabile* and *Attagenus megatoma* (Coleoptera, Dermestidae). J. Stored Prod. Res. 9:205-211.

Collins, D. 2006. A review of alternatives to organophosphorus compounds for the control of storage mites. J. Stored Prod. Res. 42:395-426.

Flores, M., C. C. Grimm, F. Toldrá, and A. M. Spanier. 1997. Correlations of sensory and volatile compounds of Spanish "Serrano" dry-cured ham as a function of two processing times. J. Agric. Food Chem. 45:2178-2186.

García, N. 2004. Efforts to control mites on Iberian ham by physical methods. Exp. Appl. Acarol. 32:41-50.

Hoy, M. A. 2011. Agricultural acarology: Introduction to integrated mite management, vol. 7. CRC Press, Boca Raton, FL.

Hughes, A. M. 1976. The mites of stored food and houses. Technical Bulletin, Ministry of Agriculture, Fisheries and Food, HMSO, London, UK.

Ignatowicz, S., Chemosterilisation of acarid mites (Acarida: Acaridae). 1982. Part I. Infecundity induced in females of the flour mite, *Acarus siro* L., by potassium iodide. *Roczniki Nauk Rolniczych* 12:11–21.

Ignatowicz, S., Chemosterilisation of acarid mites (Acarida: Acaridae). 1982. Part II. Boric acid and sodium borate effects on fecundity and longevity of *Tyrophagus putrescentiae* (Schrank). *Roczniki Nauk Rolniczych* 12:23–35.

Ignatowicz, S., Chemosterilisation of acarid mites (Acarida: Acaridae). 1986. Part IV. Potassium iodate and tin iodide as chemosterilants of the mold mite, *Tyrophagus putrescentiae* (Schrank). *Roczniki Nauk Rolniczych* 16:9–16.

Junqueira-Gonçalves, M. P., É. Alarcon, and K. Niranjan. 2013. Development of antifungal packaging for berries extruded from recycled PET. Food Control 33:455–460.

Labuza, T., and R. Contreras-Medellin. 1981. Prediction of moisture protection requirements for foods. Cereal Foods World (USA).

Lee, C., B. Sung, and H. Lee. 2006. Acaricidal activity of fennel seed oils and their main components against *Tyrophagus putrescentiae*, a stored-food mite. J. Stored Prod. Res. 42:8-14.

Lewis, S. E. 2014. Multifunctional compositions having combined insecticidal, miticidal and fungicidal activity. US Patent 8 815 303.

Macchioni, F., P. Cioni, G. Flamini, I. Morelli, S. Perrucci, A. Franceschi, G. Macchioni, and L. Ceccarini. 2002. Acaricidal activity of pine essential oils and their main components against *Tyrophagus putrescentiae*, a stored food mite. J. Agric. Food Chem. 50:4586-4588.

Marriott, N., and M. Schilling. 2004. Dry cured pork research review white paper. *In* National Country Ham Association Annu Meeting, Conover, NC.

Marriott, N. G., and H. W. Ockerman. 2004. The Ultimate guide to country ham: an American delicacy. Brightside Press, Radford, VA.

Milani, N. 2001. Activity of oxalic and citric acids on the mite *Varroa destructor* in laboratory assays. Apidologie 32:127-138.

Milne, D. 1963. A study of the nutrition of the cigarette beetle, *Lasioderma serricorne* F., (Coleoptera: Anobiidae) and a suggested new method for its control. J Entomol Soc S Afr 26:43-63.

Mueller, D. K. 2010. Reducing customer complaints in stored products. Insects Limited, Westfield, IN.

Nayak, M. K. 2006. Management of mould mite *Tyrophagus putrescentiae* (Schrank) (Acarina: Acaridae): a case study in stored animal feed. *Int Pest Control* 48:128-130.

Pankiewicz-Nowicka, D., J. Boczek, and R. Davis. 1986. Attraction by selected organic compounds to *Tyrophagus putrescentiae* (Acari: Acaridae). Ann. Entomol. Soc. Am. 79:293-299.

Qu, S. X., H. P. Li, L. Ma., L. J. Hou., J. S. Lin., J. D. Song., and X. Y. Hong. 2015. Effects of different edible mushroom hosts on the development, reproduction and bacterial community of *Tyrophagus putrescentiae* (Schrank). J Stored Prod Res 61:70–75.

Rentfrow, G., D. Hanson, M. Schilling, and W. Mikel. 2008. The use of methyl bromide to control insects in country hams in the Southeastern United States. Extension Publication. University of Kentucky Extension/National Country Ham Association. Publication# ASC-171 1-2.

Rodrigues, J. G., and M. F. Potts. **1975**. Nutritional requirements and metabolic inhibition of some acarid mites, In *Proc 1st Int Working Conf – Stored Product Entomology*, Savannah, GA, 1974. pp. 160–161.

Rodriguez, J. G. 1972. Inhibition of acarid mite development by fatty acids, *In* J. G. Rodriguez, Insect and mite nutrition. North-Holland Publishing Company, Amsterdam, Netherlands. pp. 637–650.

Samelis, J., G. Bedie, J. Sofos, K. Belk, J. Scanga, and G. Smith. 2005. Combinations of nisin with organic acids or salts to control *Listeria monocytogenes* on sliced pork bologna stored at 4 °C in vacuum packages. LWT-Food Science and Technology 38:21-28.

Sánchez-Ramos, I., and P. Castañera. 2001. Development and survival of *Tyrophagus putrescentiae* (Acari: Acaridae) at constant temperatures. Environ. Entomol. 30:1082-1089.

Sánchez-Ramos, I., and P. Castañera. 2005. Effect of temperature on reproductive parameters and longevity of *Tyrophagus putrescentiae* (Acari: Acaridae). Experimental and Applied Acarology 36:93-105.

SAS Online Document 9.1.3. 2010. SAS Institute, Cary, NC.

Stara, J., M. Nesvorna, and J. Hubert. 2014. Comparison of the effect of insecticides on three strains of *Tyrophagus putrescentiae* (Acari: Astigmata) using an impregnated filter paper test and a growth test. Pest Manag. Sci. 70:1138-1144.

Thorsteinson, A. 1960. Host selection in phytophagous insects. Annu. Rev. Entomol. 5:193-218.

United States Environmental Protection Agency. **2006**. Reregistration Eligibility Decision for Propylene Glycol and Dipropylene Glycol. Washington, DC. http://www.epa.gov/pesticides/reregistration/REDs/propylene_glycol_red.pdf [23 August 2015].

United States Environmental Protection Agency. **2015**. The Montreal protocol on substances that deplete the ozone layer, Washington, DC. http://www.epa.gov/ozone/intpol/

United States Food and Drug Administration. 2015. CFR-code of federal regulations title 21. Washington, DC. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm

Ustunol, Z., M. E. Embuscado, and K. C. Huber. 2009. Edible films and coatings for meat and poultry. *In* M. E. Embuscado, and K. C. Huber, Edible Films and Coatings for Food Applications, Springer, Dordrecht, Netherlands. pp. 245–268.

Zhao, Y., T. W. Abbar, M. W. Phillips, and Schilling. 2015. Phosphine fumigation and residues in dry-cured ham in commercial applications. Meat Sci. 107:57-63.

Table 2-1 Food additives and other compounds studied for their ability to suppress ham mite population growth on small ham cubes using topical application

Category	Compound	Title 21 CFR[a]	Supplier[b]
Salts and Free Acids	calcium propionate	184.1221	A
	sodium propionate	184.1784	A
	potassium propionate	na	B
	calcium sorbate	184.1195	A
	sodium sorbate	184.1751	A
	potassium sorbate	184.1625	A
	sodium citrate	184.1751	A
	calcium citrate	184.1195	A
	potassium citrate	184.1625	A
	sodium iodate	184.1635	A
	calcium iodate	184.1206	A
	potassium iodate	172.375	A
	sodium carbonate	184.1742	A
	malic acid	184.1069	A
	citric acid	184.1033	A
Oils/Fat	canola oil	na	C
	light-mineral oil	172.878	D
	soybean oil	na	C
	corn oil	na	C
	olive oil	na	C
	lard (pork fat)	182.70	E
Organic Alcohols	**1**-propanol	172.515	D
	2- propanol	172.515	D
	1,2 propanediol	184.1666	D
	1,3 propanediol	na	A
	1,2 butanediol	na	A
	1,3 butanediol	na	A
	glycerol	182.1320	D

Miscellaneous Additives			
	carrageenan	184.1221	F
	Propylene glycol-alginate	172.858	F
	xanthan gum	172.695	F
	butylated hydroxyanisole (BHA)	172.110	G
	butylated hydroxytoluene (BHT)	172.115	G
	natamycin	na	H
	ethoxyquin	573.380	G

[a] Specific regulation number, except where not applicable (na), from Title 21 of the United States Code of Federal Regulations (CFR 21) addressing foods an drugs (CRF)
[b] Suppliers and vendors, by US state and city (where available) for food additives used here are by letter designation: A = Acros organics, New Jersey; B = Tokyo chemical industry Co., LTD, Oregon; C = Wal-Mart Stores Inc, Arkansas; D = Fisher, Pennsylvania; E = Armour Meats, Ohio; F = Ingredients Solutions INC, Maryland; G = MP Biomedicals, California; H=DSM Food Specialities, Delft, NL.

Table 2-2 Mean (SE) number of *T. putrescentiae* fed on small dry-cured ham cubes treated with different concentrations of salts and free acids after 2 weeks (n = 5).

Salts and free acids	% Concentration	Mean number of mites (se)			*F*, df, *P*
Water control	--	312.8	(26.1)	a	18.29, 3, 12 <.0001
Calcium sorbate	1	185.2	(30.3)	b	
Calcium sorbate	5	86.2	(16.7)	c	
Calcium sorbate	10	81.4	(11.5)	c	
Water control	--	328.4	(35.8)	a	6.32, 3, 12, 0.0081
Sodium sorbate	1	202.8	(33.7)	b	
Sodium sorbate	5	212.0	(38.9)	b	
Sodium sorbate	10	122.2	(31.0)	b	
Water control	--	248.4	(35.6)	a	7.30, 3, 12, 0.0048
Calcium propionate	1	215.6	(19.7)	a	
Calcium propionate	5	195.2	(24.4)	ab	
Calcium propionate	10	99.8	(16.6)	b	
Water control	--	648.2	(69.5)	a	11.48, 3, 12, 0.0008
Sodium iodate	10	290.4	(27.8)	b	
Potassium iodate	10	277.0	(36.2)	b	
Calcium iodate	10	415.0	(42.7)	b	
Water control	--	184.4	(22.9)	b	9.11, 3, 12, 0.0020
Citric acid	10	298.4	(36.4)	a	
Maleic acid	10	97.8	(34.9)	c	
3,3 thiodipropionic acid	10	153.6	(11.1)	bc	

For each experiment, means followed by different letters are significantly different ($P < 0.05$, by LSD test)

Table 2-3 Mean (SE) number of *T. putrescentiae* fed on small dry-cured ham cubes treated with different concentrations of oils and fat after 2 weeks (n = 5).

Oils/Fat	% Concentration	Mean number of mites (se)			*F*, df, *P*
Untreated control	--	679.6	(127.0)	a	22.26, 4, 16 <.0001
Hexane control	100	453.8	(54.3)	b	
Lard % in hexane	10	462.2	(46.6)	b	
Lard % in hexane	50	166.8	(30.5)	c	
Lard % in hexane	100	3.0	(1.4)	c	
Untreated control	--	397.6	(61.4)	a	10.54, 3, 12, 0.0011
Soybean oil	100	197.0	(32.8)	b	
Light-mineral oil	100	176.0	(37.5)	b	
Canola oil	100	88.6	(8.1)	c	

For each experiment, means followed by different letters are significantly different ($P < 0.05$, by LSD test)

Table 2-4 Mean (SE) number of *T. putrescentiae* fed on small dry-cured ham cubes treated with different concentrations of organic alcohols after 2 weeks (n = 5).

Organic Alcohols	%Concentration	Mean number of mites (se)			F, df, *P*
Untreated control	--	698.8	(97.9)	a	44.56, 7, 28 <.0001
1-propanol	100	361.2	(63.0)	b	
2- propanol	100	100.6	(14.2)	c	
1,2 propanediol (propylene glycol)	100	4.6	(2.5)	c	
1,3 propanediol	100	11.0	(3.5)	c	
1,2 butanediol	100	473.8	(24.3)	b	
1,3 butanediol	100	23.0	(5.2)	c	
1,4 butanediol	100	32.6	(9.6)	c	
Water control	--	172.2	(32.2)	a	18.73, 3, 12, <.0001
Propylene glycol in water	10	122.4	(18.0)	a	
Propylene glycol in water	25	67.6	(17.6)	b	
Propylene glycol in water	50	1.0	(0.6)	c	
Water control	--	367.4	(24.2)	a	3.27, 3, 12, 0.0591
Glycerol in water	20	343.6	(25.6)	a	
Glycerol in water	50	323.2	(19.9)	ab	
Glycerol in water	100	279.2	(20.3)	b	

For each experiment, means followed by different letters are significantly different ($P < 0.05$, by LSD test)

Table 2-5 Mean (SE) number of f *T. putrescentiae* fed on small dry-cured ham cubes treated with different concentrations of food preservatives after 2 weeks (n = 5).

Food preservatives	% Concentration	Mean number of mites (se)			F, df, *P*
Acetone control	--	400.6	(24.2)	a	75.2, 2, 8, <.0001
Ethoxyquin in acetone	1	122.8	(4.3)	b	
Ethoxyquin in acetone	10	14.6	(17.4)	c	
Untreated control	--	538.4	(109.3)	a	10.30, 2, 8, 0.0061
Ethanol control	100	362.6	(25.0)	b	
BHT in ethanol	10	0.0	(0.0)		
BHA in ethanol	10	139.2	(51.8)	c	

For each experiment, means followed by different letters are significantly different ($P < 0.05$, by LSD test).

Table 2-6 Mean (SE) number of *T. putrescentiae* fed on small dry-cured ham cubes treated with different concentrations of propylene glycol with carrageenan after 2 weeks (n = 5).

	% Concentration	mean number of mites (se)			*F*, df, *P*
Water control	--	321.2	(27.1)	a	22.26, 3, 12, <.0001
Water + carrageenan	98 + 2	233.6	(32.2)	b	
PG + Carrageenan	98 + 2	3.0	(1.1)	c	
PG	100	2.0	(0.8)	c	
Carrageenan + water	2 + 98	404.8	(78.1)	a	15.47, 4, 16 <.0001
PG + carrageenan + water	5 + 2 + 93	377.0	(11.9)	ab	
PG + carrageenan + water	25 + 2 + 73	249.2	(61.9)	ab	
PG + carrageenan + water	50 + 2 + 48	216.6	(26.3)	ab	
PG	100	9.0	(2.8)	c	

For each experiment, means followed by different letters are significantly different ($P < 0.05$, by LSD test).

Table 2-7 Mean (SE) of total number of mites counted at six sampled locations (142.5 cm^2) on each of four treated hams (aged for 6 weeks) for different treatments following inoculation of each ham with approximately 900 mites.

Treatment	%Concentration	mean number of mites (se)			F, df, *P*
Untreated control	--	166.5	(27.0)	a	9.93, 6, 21, <.0001
Xanthan gum + PG	2+ 40	137.25	(79.7)	a	
Xanthan gum + PG	2+ 0	95.25	(35.8)	a	
Xanthan gum + PG	2+ 20	100.5	(85.6)	ab	
Carrageenan + PGA+ PG	1+1+0	58.5	(19.1)	ab	
Carrageenan + PGA+ PG	1+1+20	9.0	(2.6)	bc	
Carrageenan + PGA+ PG	1+1+40	7.25	(2.5)	c	

For each experiment, means followed by different letters are significantly different ($P < 0.05$, by HSD test).

Table 2-8 Mean (SE) of total number of mites counted on whole sampled area (142.5 cm^2) on each of four treated hams (aged for 12 weeks) for different treatments. Mite infestations were present through natural mite population growth from past inoculations.

Treatment	%Concentration	mean number of mites (se)			F, df, *P*
Untreated control	--	349.5	(105.2)	a	4.92, 6, 21, <.0027
Xanthan gum + PG[a]	2+ 40	274.8	(96.0)	a	
Xanthan gum + PG	2+ 0	268.3	(148.5)	a	
Xanthan gum + PG	2+ 20	209.0	(26.9)	ab	
Carrageenan + PGA[b]+ PG	1+1+0	206.0	(53.5)	ab	
Carrageenan + PGA+ PG	1+1+20	172.0	(43.2)	ab	
Carrageenan + PGA+ PG	1+1+40	79.75	(63.7)	b	

Means followed by different letters are significantly different ($P < 0.05$, by HSD test).

Chapter 3 - Efficacy of selected pesticides against *Tyrophagus putrescentiae* (Schrank): influence of applied concentration, application substrate, and residual activity over time

Abstract

Dry-cured ham is protected from arthropod infestations using the fumigant methyl bromide. *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae), known as the cheese or ham mite, decreases the quality and quantity of dry-cured hams during the aging process and is a serious economic threat to dry-cured ham companies. Methyl bromide is a strong ozone-depleting substance that is scheduled to be phased out from most uses in the near future. Consequently, developing effective, feasible, and economic alternatives to methyl bromide is the main purpose of the current project. More than ten registered residual pesticides were assessed for toxicity with glass vials treated with different concentrations. Twenty mites were transferred to each vial for 24 h of exposure on contact. Persistence of three of these residual pesticides was evaluated over a 2-month period by applying the recommended label rates to different surfaces. Results indicated that the commercial products of deltamethrin plus chlorpyrifos-methyl, chlorfenapyr, and malathion showed promising results for contact toxicity against mites, and chlorfenapyr was highly effective at very low concentrations. Chlorfenapyr was the only pesticide applied to metal, concrete, and wood that was effective at controlling ham mites for 8 weeks. These pesticides are currently registered for use in similar contexts, so they could be considered as new potential control measures for ham mites in ham plants.

Introduction

Tyrophagus putrescentiae (Schrank) (Sarcoptiformes: Acaridae), a serious pest of dry-cured ham, infests a wide range of stored and processed foods including grain, flour, and cereal products, stored seeds, cheeses, hams, and sausages (Athanassiou et al. 2011, Ayguna et al. 2007, García 2004, Athanassiou et al. 2002, Thind and Clarke 2001). American dry-cured ham, also known as country ham, is produced from the hind leg of a pork carcass by rubbing salt, sugar, and other spices, sometimes followed by a smoke treatment, and then aged between 3 and 24 months to develop desirable flavor (Marriott and Schilling 2004). Cured hams that are subjected to aging for longer than 5 months are exposed to a greater risk for infestation by *T. putrescentiae* (Rentfrow et al. 2008). Methyl bromide (MeBr, CH_3Br) is a broad spectrum fumigant that controls insects, mites, rodents, micro flora, and nematodes, and has been commercially used for more than 40 years (Duniway 2002). This fumigant has been utilized as the most effective control method against *T. putrescentiae* by dry-cured ham producers in the United States (Rentfrow et al. 2008). However, methyl bromide is classified as a significant ozone-depleting substance and has been phased out for most uses. Therefore, there is a significant need to find safe, feasible, economic, and effective alternative pest control methods for dry-cured hams.

Sanitation is a main key to avoid notable mite infestations, although, the complexity of racks in dry-cured-ham aging rooms makes it difficult to use sanitation as an effective preventive control method. All plants with aging periods less than 5 months that did not report infestation problems had aging times of around 3 months, good sanitation, and fly control (Rentfrow et al. 2008). Sanitation is necessary because ham mites will survive and thrive if facilities are not completely cleaned. However, some plants with good sanitation can still have infestations when

hams are aged longer than 5 months. Furthermore, abundant refuges such as cracks, crevices, and pathways for pests help ham mites to survive and thrive. Hence, sanitation and exclusion practices alone are not effective at controlling ham mites.

Pest-management programs for flour mills and grain storages involve contact insecticides (Athanassiou et al. 2014). Many pesticides that are used to control stored-product insects also show acaricidal activity, but research with pesticides targeted specifically at *T. putrescentiae*and other stored-product mites is limited. There are various compounds used efficiently for acarine pest control in field crops, veterinary, and public health programs (Collins 2006). Diatomaceous earth, essential oils, and bean flour have toxic effects on stored product mites, but their use is very limited (Hubert et al. 2007, Palyvos et al. 2006, Sung et al. 2004). Several pyrethroids have been identified as effective control agents against stored products mites. Populations of *Acarus siro* L. and *Lepidoglyphus destructor* (Schrank) were suppressed by over 99 % when treated with 2 ppm bifenthrin applied to grain (Collins et al. 2001). Moreover, deltamethrin and bifenthrin reduced the population growth of *T. putrescentiae* by approximately 80 % at 4 ppm applied on grain (Collins et al. 2001). Chlorfenapyr is a broad spectrum insecticide–acaricide that can control all developmental stages of phytophagous mites and various insect pests. It has moderate mammalian toxicity (LD_{50} = 441 mg/kg) and persists in the environment for a long period of time (DT_{50} > 365 days) (Dekeyser 2005). Athanassiou et al. (2014) assessed the residual effect of chlorfenapyr for control of stored-product psocid species, *Liposcelis entomophila* (Enderlein) and *L. paeta* Pearman. Chlorfenapyr caused 99–100 and 92–100 % mortality of *L. entomophila* and *L. paeta,* respectively, at rates of 13.8 mg/m^2 or higher after 3 d of exposure. A commercial combination of chlorpyrifos-methyl and deltamethrin can be directly applied on wheat and other small grains in the United States (US), with activity against several species of

stored grain insects (Arthur 2012). Effects of other pesticides such as Azadirachtin have been assessed for controlling population growth of *T. putrescentiae* with up to 100 % inhibition at certain concentrations (Collins 2003). However, no studies that evaluated deltamethrin plus chlorpyrifos-methyl for controlling stored product mites were found in our literature review.

In addition to limitations on the types of pesticides that can be used to protect stored-products (Stara et al. 2011b), another challenge of pesticide use in storage and food-processing places is the persistence of these compounds on treated surfaces. Extended residual efficacy of a pesticide is highly desirable because long-term control reduces the need to repeat the application. Several studies have tested the efficacy and persistence of contact insecticides on various surfaces that are similar to those in food-processing facilities in order to assess a pesticide's potential value in commercial application (Arthur 2008, , Arthur et al. 2009, Athanassiou et al. 2011, Wijayaratne et al. 2012, Athanassiou et al. 2013, 2014). Results obtained from these studies vary due to species susceptibility, life stage, pesticide absorption to the given surface, the material and method applied in surface preparation, persistence of pesticide related to its structure, exposure period, and aging intervals.

Therefore, the general purpose of this study was to evaluate the activities of registered pesticides from horticulture, field crops, and stored products for their potential to control *T. puterescentiae*. Specifically, initial screening studies of several active ingredients were conducted to determine compounds with potential for controlling *T. putrescentiae.* The mortality rates of mites exposed to three different surfaces treated with the most active pesticides were evaluated and treated surfaces aged up to 8 weeks post-treatment to assess residual activity.

Materials and Methods

Mite cultures

Mites used in this study were from our laboratory colony that was started with locally field-collected mites in 2010. Mites were reared in 917-ml glass canning jars and sealed with filter paper (Whatman™/cat No. 1001 090, 90 mm) within the metal lid ring. The mite-rearing diet was prepared for six rearing jars and was composed of 5 g each of agar (ICN Biomedicals, Inc.); yeast (MP Biomedicals, LLC); alphacel (ICN Biomedicals, Inc.); mixed vitamins (Vanderzant modification vitamin mixture for insect diet; MP Biomedicals, LLC); 160 g commercial dry dog food; 25 ml glycerol (Fisher); 25 ml antifungal salt solution in ethanol (methyl-p-hydroxybenzoate, 15:85 g/ml) (ICN Biomedicals, Inc.); and 475 ml tap water. The water, dog food, and glycerol were mixed and brought to a boil; other dry ingredients and the antifungal salt solution were added and cooked at a slow boil for about 30 min. The hot diet mixture was then added to each of the six jars together with about 80 g of additional dog food per jar. After the diet was cooled, approximately 16,000 mixed life stages of mites were introduced to jars that were then held at 25 °C and 70 % RH in darkness for 14–28 days, after which new mites could be harvested for experiments.

Pesticides

The pesticides tested in the experiments were commercial formulations of products available in the USA. Pesticides were selected so that a variety of chemical classes were represented and that those selected were permitted for various uses by regulatory agencies. A list of pesticides tested and their active ingredients, chemical groups, and recommended amount of active ingredient per square meter of application, is listed in Table 3-1.

Pesticide screening bioassays

Initial experiments were conducted to determine toxic activity of the pesticides against *T. putrescentiae*. A volume of 100 ml for each commercial product was made with a concentration equal to twice the EPA-recommended label rate in distilled water. This starting solution was then further diluted in half for the recommended label rate, and then again diluted 50 % to make a solution at half of the label rate. Pesticide bioassays were conducted in completely randomized designs based on methods of Yang et al. (2001), with slight modifications. Glass vials (4 ml) were coated on the inside surface with 35 µl of each pesticide solution. There were five experimental units per concentration. For a given compound, vials treated with distilled water were used as controls. Twenty unsexed adults and nymphs were transferred into each vial, and vials were sealed with screw-on plastic nonventilated lids. A small piece of wet filter paper (25 mm^2) was placed in each vial to provide humidity for mites during the bioassay. The experimental vials with mites were held for 24 h in an incubator at 25 °C, after which mites were transferred to a clean Petri dish (3 cm by 1 cm height, Fisher, PA) with 0.2 g of dog food and small piece of wet filter paper (25 mm^2) for a recovery period of 24 h before assessing mortality. A mite that did not move after contact with a brush was considered dead compared to mites that moved, which were considered alive.

Residual activity on different surfaces

To investigate the residual effect of the two most highly toxic pesticides (deltamethrin plus chlorpyrifos-methyl and chlorfenapyr) from the screening assays, 200 vials were treated with the recommended label rate of each pesticide, or distilled water as controls (n = 10), and then aged for either 1–2–4–6 or 8 weeks at ambient conditions (approximately 25 °C, 40 % R.H.) under total darkness. Twenty mites were transferred to each vial at each time period and

mortality assessed following a recovery period as described above. These bioassays showed that deltamethrin plus chlorpyrifos-methyl and chlorfenapyr caused 100 % mortality of mites assayed 8 weeks after application. Therefore, subsequent studies on residual activity were conducted using surfaces other than glass. All tests were conducted using disposable plastic Petri dishes (3 cm by 1 cm height, Fisher, PA) with a total inside surface area (bottom, top and side) of 20 cm^2. These experiments used a completely randomized design with a 4 × 6 factorial structure, of 4 different treatments and 6 post-application time periods, for each of three different surfaces. Wood, metal, and concrete were utilized as the floors of the experimental dishes in a manner similar to that of Arthur et al. (2008). The wood surface was a circular piece of 3.1-mm-thick plywood, and the metal surface was a zinc-plated washer (Home Depot, KS), with each being fitted inside the dish bottoms. Both of these surfaces were glued to the Petri dish on the bottom (Elmer, Elmer`s Products, Inc. OH) and the sides (hot glue, Adhesive Technologies, Inc. NH) so that mites only came in contact with the treated areas. The concrete surface was made using a driveway patching material (Midwest Products, IN®) mixed with water to form a thick fluid, and poured into the Petri dish bottom to create a smooth surface of about 2.5 mm in height. All surfaces were prepared 2 weeks prior to application of the pesticides. One hundred ml of the recommended label rates of deltamethrin plus chlorpyrifos-methyl, malathion, and chlorfenapyr were formulated in a glass beaker using distilled water as a diluent. Solutions were applied on the individual surfaces using a Badger 100 artists' airbrush (Badger Corporation, Franklin Park, IL). The volume of spray was 180 µl applied to all inside surfaces of each dish. Distilled water sprayed at 180 µl served as a control. There were three insecticidal treatments and a control for each of the three surfaces and six residual testing periods. Ten experimental replicates were prepared and tested for each pesticide and control treatment for each surface. The dishes were

held in storage cabinets under ambient conditions (approximately 25 °C and 40 % R.H.) until they were used for the mortality bioassays. Six post-treatment testing time intervals were 1 day, 1 week, and 2, 4, 6 and 8 weeks. Twenty unsexed adults and nymphs were placed in each dish at a given post-treatment time, and a small piece of wet filter paper (25 mm^2) was added to each Petri dish and then the dishes were placed in a glass desiccator at 85 % R.H., 25 °C, and darkness. Mites were exposed to treated surfaces for 24 h and then transferred to a clean petri dish with 0.2 g of dog food and small piece of wet filter paper (25 mm^2) for a 1-day recovery period, followed by mortality assessment as described above.

Data Analysis

The data were adjusted using Arcsine transformation (Mosteller and Youtz 1961) of the proportion killed and then submitted separately for screening experiments and aged surface experiments to one-way and two-way ANOVAs, using GLM models, respectively (SAS Institute Inc. 2010). Means were separated by the Tukey's HSD (honest significant difference) test at $P < 0.05$.

Results

Screening bioassays

Mite mortality increased significantly with increasing concentration of many of the pesticides tested, including deltamethrin plus chlorpyrifos-methyl, chlorfenapyr, malathion, and rosemary. These pesticides caused 95 % or higher mortality of adults and nymphs when applied at half the label rate (Table 3-2). Furthermore, deltamethrin plus chlorpyrifos-methyl and chlorfenapyr elicited very high mortality rates at concentrations that were much lower than the label rate. Carbaryl, gamma-cyhalothrin, and deltamethrin had greater than 60 % toxicity against mites tested in these studies. For the other five pesticides evaluated, mite mortality was less than or equal to 10 %. Mortality at 1× or 2× label rates was low and not different from untreated controls for avermectin, azadirachtin, tau-fluvalinate, and bifenazate (Table 3-2).

Residual activities on different surfaces

The overall ANOVA showed that main effects of time and pesticide, and the interaction effect of time × pesticide for all surfaces were significant at $P < 0.05$ (Table 3-3). Adult mortality for water-treated control surfaces was below 5 %, except on metal (7.5 % mortality) at 4 weeks post-treatment, and on wood (9.5 % mortality) at 1 week post-treatment.

Data for chlorfenapyr confirmed that this compound retained its efficacy to control ham mites for the entire experimental period on metal, concrete, and wood (Table 3-4). On metal, deltamethrin plus chlorpyrifos-methyl and malathion were most efficacious at 1 day post-treatment with about 80.5 and 95.5 % mortality, respectively, and then the effectiveness of these pesticides gradually decreased ($P < 0.05$) over the experimental period. Deltamethrin plus chlorpyrifos-methyl applied to concrete exhibited only 17.5 % mortality 1 day post application, and this decreased to less than 10 % for the rest of the time intervals evaluated (Table 3-4). Malathion on concrete

caused 71 % mortality at the first time interval, and then had a trend of decreased residual activity up to 6 weeks (Table 3-4). Mortality from malathion on concrete was then 51.5 % at 8 weeks, which was unexpected and surprising (Table 3-4). Malathion applied to wood also showed variable residual activity over time, with low mortality at 1 day, 1 and 2 weeks, but then an increase to 61 % at 4 weeks and then down again to less than 10 % kill for 6 and 8 weeks (Table 3-4). Results indicated that on wood, deltamethrin plus chlorpyrifos-methyl did not kill more than 20 % of mites tested at any of the given post-treatment time intervals (Table 3-4).

Discussion

Several studies have reported the efficacy of plant essential oils for the control of stored-product mites (e.g., Gulati 1997, Sánchez-Ramos and Castañera 2001, Stara et al. 2011a, Collins 2003, Song et al. 2011, Assis 2011, Jeon et al. 2014). Jeon et al. (2014) showed that *Rosmarinus officinalis* oil was highly effective (LD_{50} = 8.24 μg/cm^3) to control *T. putrescentiae*. This result was consistent with our result that showed rosemary oil caused 97 % mortality to mobile stages of *T. putrescentiae* at the recommended application rate. Commercial products of azadirachtin, a natural product from the neem tree (*Azadirachta indica*), have shown preventive control in combination with mite diet (Collins 2003, Gulati 1997). Stara et al. (2011a) indicated that neem had high toxicity against *T. putrescentiae*, *Dermatophagoides farina* (Hughes), and *D. pteronyssinus* (Trouessart). Our results were different from these earlier studies in that mite mortality was only 10 % when azadirachtin was tested. Sánchez-Ramos and Castañera (2003) reported low efficacy for azadirachtin against immature stages of *T. putrescentiae* when it was combined with mite diet, but they did not study azadirachtin applied to surfaces.

Pyrethroids and organophosphates vary in their effectiveness against stored-product mites. Variation in toxicity of a given compound in such research may be due to formulation, bioassay method, and the strain of mite species (Stara et al. 2014, Freitag and Kells 2013, Stara et al. 2011b). For instance, two formulations of chlorpyrifos-ethyl showed high and low toxicity of *T. putrescentiae* (Stara et al. 2011b). However, results from the current project demonstrated high levels of toxicity against ham mites with a diversity of compounds: malathion, deltamethrin plus chlorpyrifos-methyl, and deltamethin alone. Stara et al. (2011b) showed that single pyrethroids were ineffective (two formulations of deltamethrin) or had high LD_{90} (cyphenothrin,

permethrin, pyrethrum, and bifenthrin) against *T. putrescentiae*. However, the combination of deltamethrin with *S*-bioallethrin decreased the LD_{50} remarkably, suggesting that it was a highly effective mixture at controlling *T. putrescentiae* (Stara et al. 2011b). Our study indicated that a combination of chlorpyriphos-methyl with deltamethrin was highly toxic to *T. putrescentiae*and deltamethrin was relatively toxic to *T. putrescentiae* when applied on glass vials. In previous studies, the combination of chlorpyriphos-methyl with deltamethrin was reported to be toxic to psocids (Athanassiou et al. 2009). On the other hand, Stara et al. (2014) showed that using some formulations of deltamethrin did not decrease the *T. putrescentiae* growth and that chlorpyrifos-methyl was highly effective against *T. putrescentiae* (Stara et al. 2011b). In contrast to these results, other researcher reported that mixing 10 ppm of chlorpyrifos-methyl with wheat did not control *T. putrescentiae* after 4 weeks of storage (Nayak 2006a, 2006b).

Chlorfenapyr results from the current study suggest that this pesticide could potentially be used as part of a *T. putrescentiae* management program. Chlorfenapyr is a pyrolle insecticide that is effective at controlling stored-product insects (Athanassiou et al. 2014) and has controlled *Tribolium castaneum* (Herbst) at lower recommended label rate (Arthur 2013). Other studies have evaluated the persistent nature of chlorfenapyr to control stored-product pests on different surfaces like plywood, concrete, metal, and tile (Arthur et al. 2009, Arthur 2013, 2015). Stara et al. (2014) also showed that chlorfenapyr was highly toxic to three different strains of *T. putrescentiae*. However, according to the LD_{99}, the field strain (0.1 $\mu g/cm^2$) was most sensitive to chlorfenapyr while the sensitivity of the mite strain from dog food (2 $\mu g/cm^2$) and the laboratory strain (3 $\mu g/cm^2$) were similar. These researchers reported that the lethal doses of chlorfenapyr (LD_{99} = 0.1–3 $\mu g/cm^2$) were lower than the recommended dose for insects. Results

from the current study confirmed this. The current study reveals that chlorfenapyr was highly toxic, and the residual activity for chlorfenapyr controlled *T. putrescentiae* completely.

Variation in the susceptibility of *T. putrescentiae* to different pesticides on three surfaces may have been due to the pesticide mode of action or the loss of activity over time as a function of the surface to which the pesticide was applied. We evaluated each surface separately, since the effects of the two main factors, post-treatment time interval and pesticide, were of interest. Chlorfenapyr was persistent for the entire time period on all surfaces. The time interval effect changed significantly on metal from 1 day to 1 week and again at 6 weeks post-treatment for deltamethrin plus chlorpyrifos-methyl, as indicated by decreases from 80.5 % mortality to 42 % mortality at 1 week, and a decrease to 1.0 % after 6 weeks. Malathion mortality decreased between 1 day and 1 week, between 2 and 4 weeks, and between 4 and 6 weeks on metal. In addition, malathion performed better than deltamethrin plus chlorpyrifos-methyl at all-time intervals on metal. Time interval effect significantly changed after 1 week for deltamethrin plus chlorpyrifos-methyl and after every post-treatment interval for malathion on concrete with the exception of week 6. The wood that was used for this study was unpainted in order to mimic the condition of real ham plants, and our observation showed that persistent activity of pesticides tested on wood was quite low. In previous studies, it has been reasoned that porous surfaces like concrete affect the persistency of synthetic chemicals. However, persistence of pesticide effectiveness also depends on differences among materials such as concrete, wood, and metal (Arthur et al. 2009). Arthur et al. (2009) showed that pyriproxyfen had greater residual persistence on the painted wood (28 days) than on metal and concrete surfaces (21 days each). The results of the present work showed that chlorfenapyr controlled *T. putrescentiae* entirely up to 8 weeks. Similarly, previous studies have shown long residual activity for other compounds.

For example, azamethiphos was active against *L. bostrychophila* Badonnel for 36 weeks on steel and 24 weeks on concrete surfaces, and was active against *L. paeta* infestations for 28 weeks on a steel surface. However, this compound did not control *L. paeta*on the concrete surface and did not control *L. entomophila* infestations on either concrete or steel surfaces (Collins et al. 2000). Furthermore, it was reported that chlorpyrifos-methyl at concentration of 20 ml/L/m^2 did not prevent or control *T. putrescentae* infestations on a conveyor belt and storage floors in a food-processing plant (Nayak 2006a, 2006b).

The overall objective of our research has been to provide tools for inclusion in integrated pest-management programs to control *T. putrescentiae* after methyl bromide which is no longer available. The population growth rate of *T. putrescentiae* under ideal conditions has been estimated to increase 500fold monthly (Hughes 1976, Haines 1991). Tolerance for mite infestations in commercial dry-cured ham facilities is very low (Rentfrow et al. 2008), and methyl bromide fumigation had been used frequently to keep mite numbers low (Rentfrow et al. 2008). Use of residual pesticides like those studied here could be adopted as preventive control measures to keep mite populations low in dry-cured-ham aging rooms.

References

Arthur, F. H. 2008. Efficacy of chlorfenapyr against *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae) adults exposed on concrete, vinyl tile, and plywood surfaces. J Stored Prod Res 44:145–151.

Arthur, F. H. 2012. Lethal and sub-lethal effects from short-term exposure of *Rhyzopertha dominica* on wheat treated with storicide II®. J Pest Sci 85:261–265.

Arthur, F. H. 2013. Dosage rate, temperature, and food source provisioning affect susceptibility of *Tribolium castaneum* and *Tribolium confusum* to chlorfenapyr. J Pest Sci 86:507–513.

Arthur, F. H. 2015. Food source effect and residual efficacy of chlorfenapyr as a surface treatment on sealed and unsealed concrete. J Stored Prod Res 64:65–71.

Arthur, F. H., S. Liu, B. Zhao, and T. W. Phillips. 2009. Residual efficacy of pyriproxyfen and hydroprene applied to wood, metal and concrete for control of stored-product insects. Pest Manag Sci 65:791–797.

Assis, C. P. O. d. 2011. Toxicity of essential oils from plants towards *Tyrophagus putrescentiae* (Schrank) and *Suidasia pontifica* Oudemans (Acari: Astigmata). J Stored Prod Res 47:311–315.

Athanassiou, C., N. Palyvos, P. Eliopoulos, and G. T. Papadoulis. 2002. Mites associated with stored seed cotton and related products in Greece. Phytoparasitica 30:387–394.

Athanassiou, C. G., F. H. Arthur, and J. E. Throne. 2009. Efficacy of grain protectants against four psocid species on maize, rice and wheat. Pest Manag Sci 65:1140–1146.

Collins, P. J., M. K. Nayak, and R. Kopittke. **2000**. Residual efficacy of four organophosphate insecticides on concrete and galvanized steel surfaces against three liposcelid psocid species (Psocoptera: Liposcelidae) infesting stored products. J. Econ. Entomol. 93: 1357–1363.

Athanassiou, C. G., N. G. Kavallieratos, F. H. Arthur, and J. E. Throne. 2013. Efficacy of a combination of beta-cyfluthrin and imidacloprid and beta-cyfluthrin alone for control of stored-product insects on concrete. J Econ Entomol 106:1064–1070.

Athanassiou, C. G., N. G. Kavallieratos, F. H. Arthur, and J. E. Throne. 2014. Residual efficacy of chlorfenapyr for control of stored-product psocids (Psocoptera). J Econ Entomol 107:854–859.

Aygun, O., M. Yaman, and H. Durmaz. 2007. A survey on occurrence of *Tyrophagus putrescentiae* (Acari: Acaridae) in Surk, a traditional Turkish dairy product. J Food Eng 78:878–881.

Collins, D. A. 2006. A review of alternatives to organophosphorus compounds for the control of storage mites. J Stored Prod Res 42:395–426.

Collins, D. A. 2003. The efficacy of flufenoxuron, azadirachtin and a diatomaceous earth, when admixed with oilseed rape, against storage mite pests, pp 685–688. *In* Proceedings of the 8th Int. work. Conf. stored prod. Prot. New York, 22–26 July 2002. CAB International, Wallingford, Oxon, UK.

Collins, D., D. Armitage, D. Cook, A. Buckland, and J. Bell. 2001. The efficacy of alternative compounds to organophosphorus pesticides for the control of storage mite pests. HGCA Project Report.

Collins, P. J., M. K. Nayak, and R. Kopittke. **2000**. Residual efficacy of four organophosphate insecticides on concrete and galvanized steel surfaces against three liposcelid psocid species (Psocoptera: liposcelidae) infesting stored products. J Econ Entomol 93(4):1357–1363.

Dekeyser, M. A. 2005. Acaricide mode of action. Pest Manag Sci 61:103–110.

Duniway, J. 2002. Status of chemical alternatives to methyl bromide for pre-plant fumigation of soil. Phytopathology 92:1337–1343.

Freitag, J. A., and S. A. Kells. 2013. Efficacy and application considerations of selected residual acaricides against the mold mite *Tyrophagus putrescentiae* (Acari: Acaridae) in simulated retail habitats. J Econ Entomol 106:1920–1926.

García, N. 2004. Efforts to control mites on Iberian ham by physical methods. Exp Appl Acarol 32:41–50.

Gulati, R. 1997. Inhibitory action of neem products on *Tyrophagus putrescentiae* Schrank (Acarina: Acaridae) in wheat during storage. Ann Agric Biol Res 3(2):227–230.

Haines, C. P. 1991. Insects and arachnids of tropical stored products: their biology and identification (a training manual). Natural Resources Institute, Chatham Maritime, Kent.

Hubert, J., V. Stejskal, Z. Munzbergova, J. Hajslova, and F. H. Arthur. 2007. Suppressive potential of bean (*Phaseolus vulgaris*) flour against five species of stored-product mites (Acari: Acarididae). J Econ Entomol 100:586–590.

Hughes, A. M. 1976. The mites of stored food and houses. Technical Bulletin, Ministry of Agriculture, Fisheries and Food, London.

Jeon, J., J. Park, N. Chung, and H. Lee. 2014. Active monoterpene ketones isolated from *Rosmarinus officinalis* with fumigant and contact action against *Tyrophagus putrescentiae* (Schrank). J Food Prot 77:1355–1360.

Marriott, N., and M. Schilling. 2004. Dry cured pork research review white paper. National Country Ham Association, Inc., Lexington, pp 1–62.

Mosteller, F., C. Youtz. 1961. Tables of the freeman-tykey transformations for the binomial and poisson distributions. Biometrika 48:433–440.

Nayak, M. K. 2006a. Management of mold mite *Tyrophagus putrescentiae* (Schrank) (Acarina: Acaridae): a case study in stored animal feed. Int Pest Control 48(3):128–130.

Nayak, M. K. 2006b. Psocid and mite pests of stored commodities: small but formidable enemies. In: Proceedings of the 9th Int. work. Conf. stored prod. Prot. I. Lorini (ed), Campinas, Brazilian Post-harvest Association-ABRAPOS 15: pp 1061–1073.

Palyvos, N. E., C. G. Athanassiou, and N. G. Kavallieratos. 2006. Acaricidal effect of a diatomaceous earth formulation against *Tyrophagus putrescentiae* (Astigmata: Acaridae) and its predator*Cheyletus malaccensis* (Prostigmata: Cheyletidae) in four grain commodities. J Econ Entomol 99:229–236.

Rentfrow, G., D. Hanson, M. Schilling, and W. Mikel. 2008. The use of methyl bromide to control insects in country hams in the southeastern United States. Extension Publication. University of Kentucky Extension/National Country Ham Association. Publication #ASC-171 1–2.

Sanchez-Ramos, and P. Castanera. 2001. Acaricidal activity of natural monoterpenes on *Tyrophagus putrescentiae* (Schrank), a mite of stored food. J Stored Prod Res 37:93–101

Sanchez-Ramos, and P. Castanera. 2003. Laboratory evaluation of selective pesticides against the storage mite *Tyrophagus putrescentiae* (Acari: Acaridae). J Med Entomol 40:475–481.

SAS Online Document 9.1.3. 2010. SAS Institute. Cary, NC.

Song, H. Y., J. Y. Yang, J. W. Suh, and H. S. Lee. 2011. Acaricidal activities of apiol and its derivatives from *Petroselinum sativum* seeds against *Dermatophagoides pteronyssinus*, *D. farinae* and *Tyrophagus putrescentiae*. J Agric Food Chem 59(14):7759–7764.

Stará, J., M. Nesvorná, and J. Hubert. 2011a. The toxicity of selected acaricides against five stored product mites under laboratory assay. J Pest sci. 84:387-391.

Stará, J., V. Stejskal, M. Nesvorná, J. Plachy, and J. Hubert. 2011b. Efficacy of selected pesticides against synanthropic mites under laboratory assay. Pest Manag Sci 67:446–457.

Stará, J., M. Nesvorná, and J. Hubert. 2014. Comparison of the effect of insecticides on three strains of *Tyrophagus putrescentiae* (Acari: Astigmata) using an impregnated filter paper test and a growth test. Pest Manag Sci 70:1138–1144.

Sung, B., M. Kim, S. Lee, J. Son, and H. Lee. 2004. Acaricidal activity of essential oils derived from 10 Cupressaceae species against stored food and house dust mites. Food Sci Biotechnol 13:376–380

Thind, B., and P. Clarke. 2001. The occurrence of mites in cereal-based foods destined for human consumption and possible consequences of infestation. Exp Appl Acarol 25:203–215

United States Environmental Protection Agency. **2015.** Questions and answers about methyl bromide.

Wijayaratne, L. W., P. G. Fields, and F. H. Arthur. 2012. Residual efficacy of methoprene for control of *Tribolium castaneum* (Coleoptera: Tenebrionidae) larvae at different temperatures on varnished wood, concrete, and wheat. J Econ Entomol 105:718–725.

Yang, X., D. C. Margolies, K. Y. Zhu, and L. L. Buschman. 2001. Host plant-induced changes in detoxification enzymes and susceptibility to pesticides in the two spotted spider mite (Acari: Tetranychidae). J Econ Entomol 94:381–388.

Table 3-1 General information of examined registered pesticides to control *T. putrescentiae* at laboratory level.

Pesticide group	Active ingredients	Trade name	% Active Ingredient in Stock Solution	Applied Label Rate Active Ingredient (per m^2)	Manufacture
botanical	azadirachtin	Ornazin®	3	6.2 µg	SePRO
botanical	rosemary oil	Ecosmart®	1	2.5 µl	Ecosmart Technologies Inc
organic fatty acids	saturated fatty acid mixture:C8, C9, C10	Emerion™8000 C8910	15	-- [a]	Emery Oleochemicals
microbial	avermectin	Abamectin®	2	86.1 µg	Nufarm
microbial pro-insecticide	chlorfenapyr	Phantom®	21.4	1348.3 µg	Bayer CropScience
synthetic pyrethroid	tau-fluvalinate	Mavrik®	22.3	0.05 µg	Zeocon
synthetic pyrethroid	deltamethrin	Centynal®	4.8	145.7 µg	Wellmark International
synthetic pyrethroid	gamma-cyhalothrin	Bug Stop®	0.025	6.61^{-5} µl	Spectracide Home Barrier
synthetic pyrethroid + organophosphate	deltamethrin + chlorpyrifos methyl	Storcide II®	3.7 + 21.6	129 µg + 748 µg	Bayer CropScience
organophosphate	malathion	Malathion®	50	505 µg	Spectracide
carbamate	carabryl	Sevin®	22.5	75^{-4} µl	Garden Tech
carbazate	bifenazate	Floramite®	22.6	18.72 µg	OHP, Inc.

[a] There is no label available for this pesticide yet.

Table 3-2 Mortality of *T. putrescentiae* exposed to several pesticides at three concentrations in screening bioassay using glass vials

Pesticide	% Mortality (SE)					
	Concentration				$F_{(3, 16)}$	*P*-value*
	Control	**1/2 x Label rate**	**1 x Label rate**	**2 x Label rate**		
bifenazate	0.0 (0.0) a	0.0 (0.0) a	0.0 (0.0) a	1.7 (1.7) a	1.0	0.4
tau-fluvalinate	0.0 (0.0) a	2.5 (1.2) a	2.5 (0.6) a	6.7 (0.7) a	2.5	0.1
avermectin	2.5 (1.1) a	1.7 (1.2) a	3.3 (1.7) a	3.3 (2.1) a	0.21	0.9
azadirachtin	0.0 (0.0) a	0.0 (0.0) a	5.8 (1.5) b	10.0 (3.2) b	10.1	< 0.01
deltamethrin	1.7 (1.1) a	66.7 (3.1) b	65.7 (3.1) b	76.7 (5.7) b	89.7	< 0.01
carabryl	0.0 (0.0) a	88.0 (3.7) b	80.0 (5.5) b	87.0 (6.6) b	64.0	< 0.01
gamma-cyhalothrin	0.0 (0.0) a	71.0 (4.9) b	84.0 (4.9) b	NA[a]	108.9	< 0.01
rosemary oil	1.0 (1.0) a	96.0 (3.0) b	97.0 (3.0) b	NA	178.3	< 0.01
deltamethrin + chlorpyrifos methyl	1.0 (1.0) a	98.0 (1.2) b	97.0 (3.0) b	100.0 (0.0) b	260.0	< 0.01
malathion	1.0 (1.0) a	99.0 (1.0) bc	98.0 (2.0) c	98.0 (2.0) c	247.0	< 0.01
	Control	**0.7 g/ml**	**1.4g/ml**			
C8910	9.0 (1.9) a	100.0 (0.0) b	100.0 (0.0) b	NA[b]	1514.9	< 0.01
	Control	**1/2 x Label rate**	**1/4 x Label rate**	**1/8 x Label rate**		
deltamethrin + chlorpyrifos methyl	0.83 (1.0) a	98.0 (1.2) b	95.8 (1.6) b	96.0 (1.8) b	171.3	< 0.01
	Control	**0.01 x Label rate**	**0.1 x Label rate**			
chlorfenapyr[c]	5.0 (1.6) a	96.0 (1.0) b	99.0 (1.0) b	NA	367.2	< 0.01
	Control	**0.0025 x Label rate**	**0.005 x Label rate**	**0.01 x Label rate**		
chlorfenapyr	1.7 (1.0) a	94.9 (3.9) b	97.5 (1.0) b	99.2 (1.0) b	208.8	< 0.01

[a] NA indicates there were pesticides purchased in solution at the label rate concentration, therefore a double label rate could not be tested. Degree of freedom equals 2, 12 for these experiments.

[b] This recently approved (US EPA) pesticide did not have a recommended application rate at the time of this work

[c] Preliminary experiments with chlorfenapyr determined that 0.1 label rate killed nearly 100 % of *T. putrescentiae*, concentrations for chlorfenapyr consisted of lower label rate

* Within each row means followed by the same lower case letter are not significantly different; HSD test at P<0.05

Table 3-3 ANOVA for main effects residual exposure time and pesticide on different surfaces

Surface	Source	df	F	*P*-value
Metal				
	Time	5	99.1	< 0.001
	Pesticide	2	1458.6	< 0.001
	Time x Pesticide	10	35.5	< 0.001
Concrete				
	Time	5	41.3	< 0.001
	Pesticide	2	2453.9	< 0.001
	Time x Pesticide	10	29.1	< 0.001
Wood				
	Time	5	167.0	< 0.001
	Pesticide	2	1677.6	< 0.001
	Time x Pesticide	10	12.4	< 0.001

Table 3-4 Residual effects of chlorfenapyr, deltamethrin + chlorpyrifos methyl, and malathion for up to 8 weeks after application to wood, metal, and concrete surfaces on the mortality of *T. putrescentiae*

Surface	Pesticide	% Mortality (se) per Time Period[a]						
		1 DAY	1 Week	2 Weeks	4 Weeks	6 Weeks	8 Weeks	$F_{(5,54)}$, *P*
Metal								
	control	3.5 (1.5) Aa	2.5 (1.1) Aa	1.0 (0.7) Aa	7.5 (3.2) Ba	3.5 (2.5) Ba	3.5 (1.3) Aa	1.2, 0.3
	chlorfenapyr	100.0 (0.0) Ab	100.0 (0.0) Ab	100.0 (0.0) Ab	100.0 (0.0) Ab	100.0 (0.0) Ab	100.0 (0.0) Ab	0.0, 1.0
	deltamethrin + chlorpyrifos methyl	80.5 (1.2) Ac	38.5 (3.9) Bc	46.5 (5.3) Bc	42.0 (4.5) Bc	1.0 (0.7) Ca	3.5 (2.0) Ca	110.5, < 0.01
	malathion	95.5 (1.2) Ad	89.0 (1.9) Bd	88.5 (2.7) Bd	72.0 (5.0) Cd	29.5 (5.8) Dc	30.0 (3.6) Dc	93.3, < 0.01
	$F_{(3,36)}$, *P*	266.7, < 0.01	270.8, < 0.01	279.8, < 0.01	212.4, < 0.01	310.7, < 0.01	294.8, < 0.01	
Concrete								$F_{(5,54)}$, *P*
	control	2.0 (1.3) Aa	0.5 (0.5) Ba	0.0 (0.0) Ba	2.0 (1.1) Ba	0.5 (0.5) Ba	3.0 (1.1) Ba	1.2, 0.3
	chlorfenapyr	100.0 (0.0) Ab	100.0 (0.0) Ab	100.0 (0.0) Ac	100.0 (0.0) Ab	100.0 (0.0) Ac	100.0 (0.0) Ab	0.0, 1.0
	deltamethrin + chlorpyrifos methyl	17.5 (3.1) Ac	0.5 (0.0) Bc	2.5 (2.0) Aa	3.5 (1.1) Aa	6.5 (2.8) Ab	0.5 (0.5) Aa	15.9, < 0.01
	malathion	71.0 (4.3) Ac	36.5 (2.0) Bc	17.0 (2.5) Cb	5.0 (1.8) Dc	6.5 (3.7) Db	51.5 (5.5) Ec	111.4, < 0.01
	$F_{(3,36)}$, *P*	379.7, < 0.01	447.2, < 0.01	444.1, < 0.01	445.9, < 0.01	447.8, < 0.01	434.6, < 0.01	
Wood								$F_{(5,54)}$, *P*
	control	0.0 (0.0) Aa	9.5 (2.3) Ba	2.5 (1.1) Ca	1.5 (1.1) Ca	1.5 (1.1) Ca	3.5 (1.1) Ca	4.3, 0.4
	chlorfenapyr	100.0 (0.0) Ab	100.0 (0.0) Ab	100.0 (0.0) Ab	100.0 (0.0) Ab	100.0 (0.0) Ab	100.0 (0.0) Ab	0.0, 1.0
	deltamethrin + chlorpyrifos methyl	6.5 (2.2) Ac	16.0 (2.7) Bc	9.0 (3.5) Cc	4.0 (3.0) Ca	5.5 (2.9) Ca	3.0 (2.1) Ca	6.7, < 0.01
	malathion	17.0 (4.1) Ad	31.5 (3.3) Bd	10.5 (1.9) Cc	61.0 (6.9) Dc	7.5 (2.1) Ea	9.0 (1.0) Ec	42.8, < 0.01
	$F_{(3,36)}$, *P*	77.3, < 0.01	217.4, < 0.01	278.9, < 0.01	315.7, < 0.01	313.3, < 0.01	304.2, < 0.01	

[a] Within each row, means followed by the same upper-case letter are not significantly different ; within each column, means followed by the same lower-case letter are not significantly different; HSD test at 0.05.

Chapter 4 - Time-Mortality Relationships to Control *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae) Exposed to High and Low Temperatures

Abstract

Tyrophagus putrescentiae (Schrank) (Sarcoptiformes: Acaridae) is a widely distributed pest species that is of significant economic importance for dry-cured country hams. Methyl bromide was used for decades in country ham plants to control this pest, but now this fumigant is recognized as an atmospheric ozone-depleting compound and will be phased out for all uses in the near future. Of various chemical and nonchemical alternatives to methyl bromide, extreme temperatures are viable and straightforward nonchemical methods to control pests. This study evaluated the efficacy of high and low temperatures on mortality of mold mite in the laboratory. Ten eggs and a mixture of 40 adults and nymphs were separately exposed to different high and low temperatures, ranging from +35 to 45 °C and from −20 to +5 °C, for several periods of time. Mortality was assessed after a recovery period for each life stage. *Tyrophagus putrescentiae* eggs were found to be more tolerant to both high and low temperatures than were the mobile stages. Results showed that high temperatures from 40-45 °C killed all mites within 4 to 1 d, respectively, while −10 °C or lower killed all mites in less than 1 d. Regression analyses of mortality data as a function of exposure predicted times for achieving desired levels of mite mortality. This study suggests that extreme temperature treatment can play an important role in integrated pest management programs for dry-cured ham as an alternative to methyl bromide or other chemical treatments.

Introduction

Tyrophagus putrescentiae (Schrank) (Sarcoptiformes: Acaridae), known as the mold mite, copra mite, cheese mite, and ham mite, is a common and sometimes significant pest of stored-products in many countries (Cunnington 1976). It has been reported in stored cheese, cultivated mushrooms, dry-cured ham aging, pet food, grain, living plants, and fruits (Cunnington 1976, Hughes 1976, Nayak 2006, Qu et al. 2015, Rentfrow et al. 2008). *Tyrophagus putrescentiae* infestation is a serious problem for both European and American dry-cured ham producers (Sánchez-Ramos and Castañera 2001, Rentfrow et al. 2008). Mite infestation can make the product unacceptable and may potentially be allergic to sensitive consumers after ingestion or contact (Armentia et al. 1994). American dry-cured ham or "country ham" has a valued tradition that is well-known in the southern United States. Hams are produced by treating the hind leg of a butchered hog with dry salts and other seasonings, sometimes followed by smoking to add flavor, followed by aging for several months under ambient conditions (Flores et al. 1997). The aging process can vary between 3 to 24 months, depending on the desired flavor, with longer aged products having a more developed flavor and a higher market value (Marriott and Ockerman 2004).

Tyrophagus putrescentiae females are able to complete a generation in 8 to 21 d at suitable conditions of humidity and temperature (25 °C, > 65% RH). Up to 500 eggs can be laid during a female's life span at a favorable temperature between 25–32°C and high relative humidity between 70-90% (Boczek 1991). Mite infestations have generally been controlled by fumigation or other pesticide applications that are being reduced mostly because of environmental safety concerns and cost (Žďárková and Voráček 1993). Methyl bromide is commonly used to control mite infestations in the United States, but it is classified as a

significant ozone-depleting substance (US EPA 2016) and is being phased out for all pest control uses in the United States.

Research has been conducted on controlling *T. putrescentiae* infestations through the use of subfreezing temperature application (Eaton and Kells 2011), CO_2 and ozone fumigation (Sekhon et al. 2010), modified atmosphere packaging and improved packaging systems (Riudavets et al. 2009), sanitation (Rentfrow et al. 2006), diatomaceous earth application (Palyvos et al. 2006), low barometric pressure and certain light regimes (Žďárková and Voráček 1993), and high temperature (Fields 1992).

Physical control methods are gaining interest as fumigation alternatives, because they are compatible with organic production, leave no residues, have little or no negative effect on product quality, and are comparatively safe for workers (Hagstrum and Subramanyam 2006). Using extreme temperature is one of the simplest and most environmentally friendly methods of pest control. Heat disinfestations of grain and processing facilities have been used for many years to control stored-product insects (Fields 1992, Fields et al. 2012). Artificial cooling or freezing has also been identified as a means of pest control (Evans 1987,Hagstrum and Flinn 1994, Donahaye et al. 1995, Imai and Harada 2006, Abdelghany et al. 2010,Eliopoulos et al. 2011). Preserving stored-products between −10°C and −20°C for a short period can be an option for disinfestation of high-value products (Hagstrum and Subramanyam 2006). A few studies have addressed the ability of *T. putrescentiae* to survive high and low temperatures (Cunnington 1969, 1976, Žďárková and Voráček 1993, Eaton and Kells 2011). However, additional information on a range of cold and heat treatment conditions could be useful. Determining critical temperatures and exposure times for *T. putrescentiae* mortality under extreme temperatures will help to design an improved integrated pest management program in dry-cured

ham facilities and ensure disinfested products. This study aimed to determine the shortest effective exposure time over a range of low and high temperatures to control *T. putrescentiae* infestations.

Materials and Methods

Mite Culture

Mites were reared in glass jars (85 mm diameter, 160 mm height) containing mite diet and sealed with labeled filter paper (90 mm, Fisher Scientific, Pittsburgh, PA) in the metal lid ring. The rearing diet was a combination of approximately 80 g of dry whole dog food kernels per jar and 100 ml of thick gravy prepared from cooked dog food with water and other ingredients described below. To prepare the liquid part of the diet for six rearing jars, dog food (160 g) was mixed with 475 ml tap water and heated to a boil. Then, 5 g each of agar (Mooragar, Incorporated Loomis, CA), yeast (MP Biomedicals, LLC, Solon, OH), alphacel (MP Biomedicals, LLC, Solon, OH), and mixed vitamins (Vanderzant modification vitamin mixture for insect diet; MP Biomedicals, LLC, Solon, OH), and 25 ml of glycerol (Fisher Scientific, Pittsburgh, PA) were added to the mixture and cooked to a boil again. An antifungal agent (25 ml of methyl-*p*-hydroxybenzoate in ethanol, 15:85 $g.ml^{-1}$; ICN Biomedicals, Inc. Aurora, OH) was added to the mixture and it was cooked for 30 min. Approximately 500 ml of the mixture was then added to 80 g of dry whole dog food in each rearing jar and allowed to cool prior to inoculation with mites. *Tyrophagus putrescentiae* cultures have been maintained at 25°C and 70% RH in darkness at our laboratory at Kansas State University for more than 4 years and they have never been exposed to extreme temperatures (< 5°C or >40°C).

High and Low Temperatures Treatment Assay

All experiments with extreme temperatures used a standard experimental unit comprising a 4-ml glass vial (Fisher Scientific, Shell vial, 12 × 35 mm, Pittsburgh, PA) with a plastic cap modified with a 2-mm-diameter hole for ventilation that secured a fine fabric mesh (125 μm) screen to retain mites in the vial. Each vial had a small piece of dog food (200 mg), and either 10

mite eggs or a group of 40 mobile mites comprising adults and large nymphs. For heat treatment, five vials were exposed to 30, 35, 38, 40, 42, and 45 °C for 0.5 to 96 h in a laboratory oven (Imperial III, Lab Line Instruments, INC. IL). For the cold treatment, five vials were exposed to temperatures of −20, −10, −7, −5, −3, 0, and 5 °C for 0.25 to 192 h. Bioassays were conducted separately for each temperature and life stage in this study. Vials were placed into a closed Styrofoam box that was then put inside a typical home freezer (Criterion, 5.0 cu. Ft. chest freezer, Menards, WI) with a maximum freezing temperature of −22 °C. A hair-curling iron (Remington, 1" 2-in-1 Curling Iron, Walmart, KS) was in the Styrofoam box and controlled by a thermostat (Fisher Scientific, Temperature controller with timer, Pittsburgh, PA) that allowed us to set the desired temperature above −22 °C by heating the air inside the box. The timer started when the freezer door was closed, so that the time taken to reach the desired temperature was included as part of the exposure duration. Some mite mortality may therefore have occurred before the desired temperature was achieved. The relative humidity inside the jars in the oven and freezer was approximately 90% and 30%, respectively, which are the conditions one would expect in a treated building under these temperatures. Temperatures and relative humidity were monitored and recorded at 5-min intervals with HoBo data loggers (Oneset, Bourne, MA) that were placed on the shelves of the oven or inside the Styrofoam box in the freezer. An experimental control set of vials was held at 25 °C and 70% RH in a growth chamber. There were five experimental units of mobile stages and nine experimental units of eggs for each time–temperature combination. After exposure, the mobile stages and eggs in vials were held under control conditions until their viability was determined 3 d after treatments. Mortality of eggs was based on those that failed to hatch. For mobile stages, the criterion for viability assessment was any movement following physical stimulation.

Data Analysis

The 50 and 99% lethal times ($LT_{50,}$ and LT_{99}) were calculated using a complementary log-log (CLL) regression model, using SAS software, in which percentage mortality (y) was transformed to the $\log_e(\_\log_e[1 \_ y/100])$ scale, and exposure time (x) was transformed to the $\log_{10}$ scale. The goodness-of-fit of the CLL model to the data was compared using a chi-square statistic (SAS Institute Inc. 2010, Robertson and Preisler 1992).

Results

The times required to reach target high temperatures in our treatment chambers after adding mites ranged from 35 min for 30 °C and 240 min for 45 °C. Times for reaching target cold temperatures in our freezer ranged from 150 min for −20 °C to 50 min for 5 °C. The times required to achieve different levels of mortality, as percent of test groups killed, under high and low temperatures are reported in Table 4-1. In all cases, the time required to kill eggs was longer than the times required for killing the mobile stages, the adults and nymphs, of *T. putrescentiae* at the same temperature. Control mortality at 25 °C for mobile stages ranged between 0 to 2.5% and for egg mortality from 0 to 5.6%. Mortality of all mite stages was less than 100% at times of 72 h or longer in experiments with 38, 35, 30, 5, 0 °C, and −3 °C; these data were not included in further analyses in this study. Mortality of 100% of mites was achieved at 45 °C for mobile stages at 4 h. Eggs required 21 h to achieve 100% mortality at the same temperature. Time for 100% kill at the extreme cold temperature of −20 C was 2 h for the mobile stages and 12 h for eggs. At intermediate temperatures such as 42, 40, −5, −7 °C, and −10 °C, 100% mortality of eggs was achieved at times ranging between 21 h and 96 h.

Results of regression analyses for estimations of the times required to kill a certain percentage of mites at different temperatures are given in Tables 4-2 and 4-3 for mobile stages and eggs, respectively. Chi-square values were not significant ($P > 0.05$) for accurately predicting mortality at 42 °C and −20 °C for the mobile stages and at all temperatures studied for egg mortality, indicating a generally good fit of data to the CLL models (Tables 4-2 and 4-3). Significant chi-square values ($P < 0.05$) for the remaining time–mortality curves showed that the responses of *T. putrescentiae* stages were heterogeneous among the data sets. This heterogeneity can be a result of different life stages, sex, and extreme temperature acclimation in the treatment

vials, and this will need further study. The upper confidence levels of the LT_{99} estimates from the regression analyses concur nicely with times at which we observed 100% mortality in our direct experiments with a given temperature or life stage.

Discussion

The extreme temperatures that we evaluated were well beyond optimal conditions for development of *T. putrescentiae*, and our results provide good information for the development of nonchemical controls for this pest. *Tyrophagus putrescentiae* eggs were more tolerant to extreme temperatures than mobile stages, including adults and large nymphs. Results also indicated that *T. putrescentiae* were more susceptible to extreme cold temperatures, requiring shorter exposure times, than extreme high temperatures. The extreme high and low temperatures killed eggs and mobile stages in 2 d or less, and lethal temperatures just within the extreme high of 45 °C and low of −20 °C resulted in good mortality within reasonable time periods.

Previous studies investigated the effects of low or high temperatures on the mortality of *T. putrescentiae*and other stored-food mites. Cunnington (1976) found that *T. putrescentiae* adults could survive for up to 7 months at 5 °C, and females laid on average just four eggs within that time period. In addition, Cunninton (1976) showed that none of several hundred new eggs hatched, nor did immature mites progress in their development, when held at 5 °C for 7 months. Žďárková and Voráček (1993) found that adults of *T. putrescentiae* were killed at −15 °C after 30 min, but only 10% mortality was recorded after 1 h after exposure to either −5 or 0 °C.

There are no studies to our knowledge explaining the effects of cold or heat acclimation on survival of *T. putrescentiae* under short-term pest mitigation treatments. Eaton and Kells (2011) showed the super-cooling point of *T. putrescentiae* ranged from −24 to −26 °C for adults and nymphs and was −35.6 °C for eggs, but they did not determine if gradual decreases in temperature from ambient would provide acclimation such that the supper-cooling point would be lowered. However, studies on other arthropod species indicated that cold acclimation can

increase the survival of, for example, *Cryptopygus antarcticus* (Willem) (Collembola: Entomobryidae) (Worland and Convey 2001) and *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae) (Khodayari et al. 2013). Furthermore, sugar alcohols (polyols) are well-known for accumulating in cold acclimated arthropods, and such accumulation may make them more cold-tolerant (Cannon and Block 1988). Nevertheless, using extreme temperatures for short-term applications against mold mites, which involves a rapid increase or decrease in temperature to meet a target killing temperature, is unlikely to be affected by any previous acclimation of mites in the pest population within their stored-product habitats.

A review of past literature on stored-product mite susceptibility to extreme temperatures reveals that different research groups report contrasting results for similar experiments. In contrast to our finding that eggs of *T. putrescentiae* are most tolerant of both high and low extreme temperatures compared with other life stages, Eaton and Kells (2011) reported that eggs were the most cold-susceptible life stage for their experiments at −5 and −10 °C. Cunnington (1984) showed that eggs of *Acarus siro* (L.) (Sarcoptiformes: Acaridae) were all killed in 30 min at −15 °C, while Žďárková and Voráček (1993) found that 1.5 h at −15 °C applied to eggs of the predator *Cheyletus eruditus* (Prostigmata: Cheyletidae) (Schrank) resulted in substantial mortality. Further, Pulpán and Verner (1959) found that mixed life stage *A. siro* and *C. eruditus*treated with −15 and −10 °C, respectively, caused complete mortality after 48 h, but Sinha (1964) reported that *A. siro* and *Lepidoglyphus destructor* (Schrank) (Sarcoptiformes: Glycyphagidae) were controlled at 72 and 196 h, respectively, at −18 °C. Hubert et al. (2010) showed that temperatures for optimal oviposition and development varied across stored-product mite taxa, though it is unknown if such physiological differences could contribute to difference in lethal conditions from extreme temperatures across taxa and within species. The

studies on lethal temperatures discussed above used different experimental procedures for heating, cooling, and exposure times, all applied to different mite species or different strains within species on different mite diets, any or all of which may have contributed to variation found in lethal effects from extreme temperatures.

A key limitation for the development of a cost-effective extreme temperature pest mitigation method is the time and/or temperature required for killing the most tolerant life stages. Results demonstrated that eggs are the most heat-tolerant stage of *T. putrescentiae*, which is consistent with research reported by Cunnington (1976) for *T. putrescentiae* and adds to the data on heat tolerance of mite eggs reported by Žďárková and Voráček, (1993) for *A. siro* and *C. eruditus*. Heat tolerance of eggs was demonstrated much earlier for *A. siro*, *L. destructor*, and *C. eruditus* by Pulpan and Verner (1959). Heat treatment has been used to control insect pests in stored-products with attention to the most tolerant life stage. Mahroof et al. (2003) showed that young larvae of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) were the most tolerant life stage at 50˚C, while eggs, adults, and pupae required less time to kill at this temperature. *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) pupae were the most heat- and cold-tolerant life stage, compared with the adults, eggs, and larvae, in work by Loganathan et al. (2011).

The research reported here provides useful data to develop practical temperature treatments (ºC) to control populations of *T. putrescentiae.* Despite variation in the most tolerant life stages of insects and mites discussed above, all concur that similar levels of mortality can be achieved either at the most extreme temperatures for short time periods or at less extreme temperatures for longer times. However, using these temperatures or long exposure times may not result in the most cost-effective application for controlling *T. putrescentiae* in the dry-cured

ham or processed food industries. Our data for killing eggs of *T. putrescentiae* indicate that adequate control should be achieved at temperatures of 42 to 45ºC or temperatures of −10 or −20ºC in less than 48 hr. Cold temperatures for controlling mites in large rooms or buildings is not practical, though can be effective for killing mites on or inside hams, or mites contaminating other materials, by placing these inside commercial freezers. Heat treatments for buildings are commercially viable and have been applied to food-processing facilities in recent years with no reports of structural damage (Fields et al. 2012). Additional research should be conducted to determine critical hold time–temperature combinations for killing mites inside food products (i.e., aged hams), and the effect of temperature–time treatments on product quality. The ongoing reduction and eventual cessation of methyl bromide fumigation in most post-harvest contexts is facilitating adoption of various nonchemical control methods for mites, and heat and cold treatments represent viable alternatives.

References

Abdelghany A. Y., Awadalla S. S., Abdel-Baky N. F., El-Syrafi H. A., Fields P. G. 2010. Effect of high and low temperatures on the drugstore beetle (Coleoptera: Anobiidae). J. Econ. Entomol. 103: 1909–1914.

Armentia A., Fernandez A., Perez-Santos C., de la Fuente R., Sanchez P., Sanchis F., Mendez J., Stolle R. 1994. Occupational allergy to mites in salty ham, chorizo and cheese. Allergol. Immunopathol. (Madr). 22: 152–154.

Boczek J. 1991. Mite pests in stored food, In Ecology Management of Food-Industry. Pest Association Off. Anal. Chem. Arlington, Virginia. pp. 57–79.

Cannon R. J. C., Block W. 1988. Cold tolerance of microarthropods. Biol. Rev. 63: 23–77.

Cunnington A. M. 1969. Physical limits for complete development of the copra mite, *Tyrophagus putrescentiae* (Schrank) (Acarina, Acaridae), In Proceedings. 2nd International Congress of Acarology. 19-25 July 1967, Sutton Bonington, England. pp. 241–48.

Cunnington A. M. 1976. The effect of physical conditions on the development and increase of some important storage mites. Ann. Appl. Biol. 82: 175–201.

Cunnington A. M. 1984. Resistance of the grain mite *Acarus siro* L. (Acarina: Acaridae) to unfavourable physical conditions beyond the limits of its development. Agric. Ecosyst. Environ. 11: 319–339.

Donahaye E. J., Navarro S., Rindner M. 1995. Low temperature as an alternative to fumigation for disinfecting dried fruit from three insect species. J. Stored Prod. Res. 31: 63–70.

Eaton M., Kells S. A. 2011. Freeze mortality characteristics of the mold mite *Tyrophagus putrescentiae*, a significant pest of stored products. J. Econ. Entomol. 104: 1423–1429.

Eliopoulos P. A., Prasodimou G. Z., Pouliou A. V. 2011. Time-mortality relationships of larvae and adults of grain beetles exposed to extreme cold. Crop Prot. 30: 1097–1102.

Evans D. E. 1987. The survival of immature grain beetles at low temperatures. J. Stored Prod. Res. 23: 79–83.

Fields P. G. 1992. The control of stored-product insects and mites with extreme temperatures. J. Stored Prod. Res. 28: 89–118.

Fields P., Subramanyam B., Hulasare R. 2012. Extreme temperatures, In Hagstrum et al (eds), Stored-product protection. S156. Kansas State University Research and Extension publication, Manhattan, KS, USA. pp. 179–190.

Flores M., Grimm C. C., Toldrá F., Spanier A. M. 1997. Correlations of sensory and volatile compounds of Spanish "Serrano" dry-cured ham as a function of two processing times. J. Agric. Food Chem. 45: 2178–2186.

Hagstrum D. W., Flinn P. W. 1994. Survival of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in stored wheat under fall and winter temperature conditions. Environ. Entomol. 23: 390–395.

Hagstrum D. W., Subramanyam B. 2006. Fundamentals of stored-product entomology. American Association of Cereal Chemists, International, St. Paul, Minnesota.

Hubert J., Pekár S., Nesvorná M., Šustr V. 2010. Temperature preference and respiration of acaridid mites. J. Econ. Entomol. 103: 2249–2257.

Hughes A. M. 1976. The mites of stored food and houses. Tech. Bull. Minist. Agric. Fish. Food.

Imai T., Harada H. 2006. Low-temperature as an alternative to fumigation to disinfest stored tobacco of the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae). Appl. Entomol. Zool. 41: 87–91.

Khodayari S., Moharramipour S., Larvor V., Hidalgo K., Renault D. 2013. Deciphering the metabolic changes associated with diapause syndrome and cold acclimation in the two-spotted spider mite *Tetranychus urticae*. PLoS One. 8: e54025.

Loganathan M., Jayas D. S., Fields P. G., White N. D. G. 2011. Low and high temperatures for the control of cowpea beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in chickpeas. J. Stored Prod. Res. 47: 244–248.

Mahroof R., Subramanyam B., Throne J. E., Menon A. 2003. Time-mortality relationships for *Tribolium castaneum* (Coleoptera: Tenebrionidae) life stages exposed to elevated temperatures. J. Econ. Entomol. 96: 1345–1351.

Marriott N. G., Ockerman H. W. 2004. The Ultimate Guide to Country Ham: An American Delicacy. Brightside Press.

Nayak M. K. 2006. Management of mould mite *Tyrophagus putrescentiae* (Schrank) (Acarina: Acaridae): a case study in stored animal feed. Int. Pest Control. 48: 128–130.

Palyvos N. E., Athanassiou C. G., Kavallieratos N. G. 2006. Acaricidal effect of a diatomaceous earth formulation against *Tyrophagus putrescentiae* (Astigmata: Acaridae) and its predator *Cheyletus malaccensis* (Prostigmata: Cheyletidae) in four grain commodities. J. Econ. Entomol. 99: 229–236.

Pulpán J., Verner P. H. 1959. The mites of the stored grain and their control (Acari). Bohem Cent a. 1: 169–292.

Qu S. X., Li H. P., Ma L., Hou L. J., Lin J. S., Song J. D., Hong X. Y. 2015. Effects of different edible mushroom hosts on the development, reproduction and bacterial community of *Tyrophagus putrescentiae* (Schrank). J. Stored Prod. Res. 61: 70–75.

Rentfrow G., Hanson D. J., Schilling M. W., Mikel W. B. 2006. Methyl bromide use to combat mite infestation in dry-cured ham during production. 2006 Annu. Int. Res. Conf. Methyl Bromide Altern. Emiss. Reductions, 6-9th November, 2006, Orlando, Florida.

Rentfrow G., Hanson D. J., Schilling M. W., Mikel W. B. 2008. The use of methyl bromide to control insects in country hams in the Southeastern United States. Ext. Publ. Kentucky Extension/National Ctry. Ham Assoc. ASC-171: 1–2.

Riudavets J., Castañé C., Alomar O., Pons M. J., Gabarra R. 2009. Modified atmosphere packaging (MAP) as an alternative measure for controlling ten pests that attack processed food products. J. Stored Prod. Res. 45: 91–96.

Robertson J. L., Preisler H. K. 1992. Pesticide bioassays with arthropods CRC Press, Boca Raton, FL.

Sánchez-Ramos I., Castañera P. 2001. Development and survival of *Tyrophagus putrescentiae* (Acari: Acaridae) at constant temperatures. Environ. Entomol. 30: 1082–1089.

SAS Online Document 2010. PROC User's manual, version 11th ed. SAS Institute. Cary, NC.

Sekhon R. K., Schilling M. W., Phillips T. W., Aikins R. M. J., Hasan M. M., Nannapaneni R., Mikel W. B. 2010. Effects of carbon dioxide and ozone treatments on the volatile composition and sensory quality of dry-cured ham. J. Food Sci. 75: 452–458.

SAS Online Document 9.1.3. 2010. PROC User's manual, version 11th ed. SAS Institute. Cary, NC.

Sinha, R. N. 1964. Effect of low temperature on the survival of some stored products mites. Acarologia. 6: 336–341.

United States Environmental Protection Agency. **2016.** Phase-out of class I ozone-depleting substances. (https://www.epa.gov/ods-phaseout/phaseout-class-i-ozone-depleting-substances).

Worland M. R., Convey P. 2001. Rapid cold hardening in Antarctic microarthropods. Funct. Ecol. 15: 515–524.

Žďárková E., Voráček V. 1993. The effects of physical factors on survival of stored food mites. Exp. Appl. Acarol. 17: 197–204.

Table 4-1 Mean percent mortality (SE) of *T. putrescentiae* at various exposure times exposed to extreme high and low temperatures.

Temp. (°C)	Mobile stages		Eggs	
	Exp. time (h)	Mean % Mortality (SE)	Exp. time (h)	Mean % Mortality (SE)
45	0.5	15.0 (2.2)		
	1	39.5 (7.4)	1	7.8 (2.8)
	2	80.5 (5.2)		
	2.5	91.5 (5.6)		
	3	98.5 (1.0)	3	28.9 (2.0)
	4	100.0 (0.0)		
			6	73.3 (4.1)
			9	82.2 (5.2)
			12	86.7 (3.3)
			15	91.1 (3.5)
			18	9.8 (1.5)
			21	100.0 (0.0)
42	3	14.5 (0.9)		
	6	23.5 (1.3)	6	16.2 (1.9)
	9	31.5 (2.6)	9	29.9 (5.8)
	12	45.5 (2.9)	12	46.9 (5.1)
	15	52.0 (2.9)	15	42.8 (4.8)
	18	66.5 (2.3)	18	58.7 (5.8)
	21	85.0 (3.5)	21	76.8 (7.9)
	24	96.0 (2.30	24	88.2 (8.7)
	36	100.0 (0.0)	36	92.9 (7.1)
			48	94.2 (5.8)
40			12	13.3 (6.2)
	18	23.0 (3.8)	18	23.3 (3.7)
			21	27.8 (4.0)
	24	25.0 (1.8)	24	40.0 (4.1)
	28	35.5 (3.9)		
	32	58.0 (6.4)		
	36	72.5 (4.5)	36	62.2 (4.3)
	40	99.0 (0.6)		
	48	100.0 (0.0)	48	75.6 (4.1)
			72	94.4 (2.3)
			96	100.0 (0.0)

	3	14.5 (1.8)	3	10.0 (3.3)
	6	18.0 (1.7)	6	15.6 (4.5)
	9	39.5 (1.8)	9	21.1 (5.1)
-5	12	66.0 (2.6)	12	28.9 (4.2)
	15	74.0 (8.5)	15	35.6 (6.5)
	18	93.0 (4.0)	18	46.7 (6.0)
	21	100.0 (0.0)	21	55.6 (2.9)
			24	66.7 (6.0)
			36	82.2 (6.0)
			40	92.2 (2.8)
			48	100.0 (0.0)
	2	14.0 (1.0)		
	3	26.5 (5.8)	3	6.7 (2.9)
	6	38.5 (4.5)	6	17.8 (4.7)
	9	64.5 (4.6)	9	31.1 (5.1)
-7	12	77.5 (3.3)	12	38.9 (4.2)
			15	44.4 (5.6)
	21	100.0 (0.0)	21	66.7 (5.3)
			24	85.6 (4.7)
			36	91.1 (2.1)
			40	100.0 (0.0)
	1	26.0 (5.1)		
	2	38.5 (4.0)		
	3	66.0 (2.0)	3	27.8 (4.3)
	4	78.0 (2.2)		
	5	89.0 (1.3)		
-10	6	100.0 (0.0)	6	58.9 (4.2)
			9	63.3 (3.7)
			12	78.8 (4.6)
			15	88.9 (3.5)
			18	94.4 (2.4)
			21	100.0 (0.0)
	0.25	9.5 (2.0)		
	0.5	16.0 (1.7)		
	0.75	58.5 (2.5)		
	1	98.0 (0.9)	1	50.0 (5.0)
-20	2	100.0 (0.0)		
			3	60.0 (4.1)
			6	75.6 (2.9)
			9	96.7 (2.4)
			12	100.0 (0.0)

Table 4-2 Lethal time in hours to kill 50 and 99% of the mobile stages of *T. putrescentiae* exposed to different constant high and low temperatures.

Temp °C	N[a]	LT_{50} (95% CL) (h)	LT_{99} (95% CL) (h)	Slope (SE)	Intercept (SE)	χ^2	df	*P*-value
45	1400	1.23 (1.10-1.35)	3.57 (3.21-4.07)	4.09 (0.30)	-0.73 (0.11)	54.88	32	0.01
42	2000	13.72 (12.56-14.65)	32.77 (30.00-37.07)	5.01 (0.46)	-6.06 (0.59)	59.09	48	0.13
40	1600	29.79 (27.27-31.57)	48.28 (44.75-55.66)	9.04 (1.22)	-13.68 (1.91)	125.96	38	0.01
-5	1600	10.57 (9.09-11.64)	22.60 (20.47-26.43)	5.74 (0.74)	-6.24 (0.86)	102.05	38	0.01
-7	1600	6.01 (4.74-7.11)	25.91 (22.55-31.26)	2.98 (0.29)	-2.69 (0.34)	73.91	38	0.01
-10	1400	2.27 (2.01-2.50)	7.62 (6.71-9.03)	3.60 (0.29)	-1.65 (0.17)	44.89	28	0.02
-20	1200	0.72 (0.69-0.75)	1.04 (1.00-1.11)	11.86 (1.05)	1.31 (0.12)	19.09	23	0.70

[a] N= total number of individuals used for each time-mortality experiment.

Table 4-3 Lethal time in hours to kill 50 and 99% of the eggs of *T. putrescentiae* exposed to different constant high and low temperatures.

Temp. °C	N[a]	LT_{50} (95% CL) (h)	LT_{99} (95% CL) (h)	Slope (SE)	Intercept (SE)	χ^2	df	*P*-value
45	810	4.03 (3.13-4.87)	21.15 (18.47-25.06)	2.63 (0.23)	-1.96 (0.55)	78.63	79	0.49
42	900	13.92 (12.58-15.03)	33.13 (29.69-38.75)	5.03 (0.51)	-6.12 (0.66)	56.72	79	0.97
40	810	32.19 (29.23-34.99)	89.54 (79.28-104.66)	4.26 (0.33)	-6.79 (0.54)	63.8	70	0.68
-5	1080	20.26 (18.05-22.17)	55.59 (49.18-65.76)	4.32 (0.40)	-6.01 (0.59)	115.37	97	0.10
-7	990	14.03 (12.14-15.65)	49.25 (43.50-58.20)	3.47 (0.31)	-4.35 (0.43)	100.85	97	0.37
-10	720	6.26 (5.27-7.13)	24.74 (21.70-29.46)	3.17 (0.28)	-2.89 (0.31)	56.78	70	0.87
-20	540	1.66 (1.13-2.15)	18.60 (13.79-29.29)	1.80 (0.22)	-0.76 (0.16)	43.61	43	0.44

[a] N= total number of individuals used for each time-mortality experiment.

Chapter 5 - Combination of sulfuryl fluoride with heat to control the ham mite, *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae), a serious pest of country ham

Abstract

This work investigated the feasibility of using sulfuryl fluoride (SF) as an alternative to methyl bromide to control the ham mite, *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae). The combination of SF and heat was used to determine the lowest effective temperature with the shortest exposure time and lowest concentration-by-time product (CTP) of SF to control mites within the US EPA label rate of 1500 $gh.m^{-3}$. Mites were controlled at temperatures greater than 30ºC. More than one day exposure was required to kill mobile stages and eggs at 40ºC with an acceptable CTP of SF. When mites were exposed to 300 or 1000 $gh.m^{-3}$ of SF at 40ºC, the mobile stages were all killed after 36 h with 300 $gh.m^{-3}$ and at 3 h with 1000 $gh.m^{-3}$. Egg mortality at 300 $gh.m^{-3}$ was 98.9% after 96 h and 97.8% after 48 h when exposed to 1000 $gh.m^{-3}$. Eggs were very tolerant to SF. Applying heat with SF under the maximum allowable CTP will increase the efficacy of fumigant against the eggs. However, when the target CTP of SF for eggs was either 300 $gh.m^{-3}$ or 1000 $gh.m^{-3}$ at longer exposure times, mortality was not as high as with SF at 1400 $gh.m^{-3}$.

Introduction

Dry-cured hams are valuable processed meat products that are obtained from the hind leg of a hog carcass. A typical American dry-cured ham is cured with salt, sugar and other spices under refrigeration (4ºC) for approximately 6 weeks, after which it is aged for six to 24 months. A cured ham loses at least 18% of its original weight during the curing and aging processes and obtains a unique flavor from this process. The nutrient composition, water activity and flavor characteristics make dry-cured hams a suitable food for various pests such as mites and beetles. (Renfrow 2008).

Tyrophagus putrescentiae (Schrank) (Sarcoptiformes: Acaridae) infests dry-cured ham through the aging process. *T. putrescentiae,* commonly known as ham mite or cheese mite, is an invasive species to cheese, dry meat, pet food, and grain (Thind and Clarke 2001, Thind 2005, Rentfrow et al. 2008). Ham mites reproduce rapidly and complete a generation within 8 to 21 days under ideal growth conditions. The average fecundity rate of females is four eggs/day at 25ºC and 90% R.H. (Boseck 1991), which results in a high population buildup. Mites that reproduce and penetrate inside the ham can considerably decrease the economic value of dry-cured ham (Zd'árková 1991). In highly infested ham plants, massive mite populations are visually detectible on ham and rack surfaces (Zhao et al. 2016).

Methyl bromide (MB) is a very effective fumigant that has been used for decades to control ham mites in the US (Marriott and Schilling, 2004). However, it is classified as a significant ozone depleting substance under the Montreal Protocol (US EPA 2006). Therefore, MB is scheduled to be phased out for all industries in US and consequently, many MB alternatives, including sulfuryl fluoride (SF) and extreme temperatures, have been explored (Fields 1999, Boina et al. 2008, Athanassiou et al. 2012).

SF is a colorless, odorless and non-flammable fumigant that was registered in US for use in stored-products in 2005 (Reichmuth et al. 1997, Phillips et al. 2012). Compared to MB and hydrogen phosphide, SF is a non-ozone depleting and non-corrosive gas that has a faster diffusion rate than MB (Navaro 2006, Baltaci et al. 2009). A satisfactory SF application can be achieved by using an adequate concentration (C) of SF for a determined exposure time (T). Therefore, the SF application label rate is a product (P) of C and T (CTP). The US Environmental Protection Agency (EPA) established a maximum application dosage of SF use on stored-products, including ham, at a CTP of 1500 $gh.m^{-3}$.

Previous studies showed that SF controlled the post-embryonic stages of stored-product pests such as the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae); Mediterranean flour moth, *Ephestia kuheniella* Zeller (Lepidoptera: Pyralidae); cigarette beetle, *Lasioderma serricornne* (F.) (Coleoptera: Anobiidae), and other beetles and moths (Bell and Savvidou 1999, Reichmuth et al. 2003, Small 2007, Athanassiou et al. 2012). However, stored-product insect eggs are more difficult to control with SF than post-embryonic life stages. Phillips et al. (2008) studied SF efficacy for control of *T. putrescentiae* life stages at 23°C, a typical temperature in ham-aging rooms, during experimental fumigation. They estimated the lethal concentration of SF needed to kill 95% of mite eggs was about 90 $g.m^{-3}$ SF at 23°C after 48 h, which is equivalent to a CTP of 4320 $gh.m^{-3}$, or nearly 3-fold higher than the maximum allowable CTP. It was hypothesized that a higher fumigation temperature would improve the efficacy of SF against *T. putrescentiae* and lower the CTP.

Potential solutions studied for improving the mortality of insect eggs can be the use of SF at higher concentrations, in combination with heat, modified atmosphere or/and other fumigants such as hydrogen cyanide (Su and Scheffrahn 1990, Bell and Drinkall 2000, Reichmuth et al.

2003, Reichmuth and Klementz 2008, Baltaci et al. 2009, Emekci 2010). Temperature has a considerable impact on the fumigation efficacy for insect control. Higher temperature increases the insects metabolism and respiration and consequently insects intake more fumigant (Fields et al. 2012). Similarly, using heat in combination with SF may allow for a lower SF concentration to control post-embryonic stages of stored-product insect pests. Bell et al. (1999) and Reichmuth et al. (2003) reported that increased temperatures reduced the amount of SF needed to control *E. kuehniella*, *T. castaneum, Tenebrio molitor* L. (Coleoptera: Tenebrionidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) adults. *T. putrescentiae* eggs were not controlled using SF at the EPA label rate with a treatment temperature of 23°C. The objective of the research reported below was to investigate the efficacy of combining SF with high temperatures to control *T. putrescentiae* as a fumigation alternative to using MB.

Materials and Methods

Mites

Mite cultures

Mites were reared on a diet that was a mixture of approximately 80 g of dry whole dog food kernels per jar and 100 ml of thick gravy. The gravy ingredients for six rearing jars included dog food (160 g), 475 ml tap water, 5 g of each of agar (Mooragar, Incorporated Loomis, CA), yeast (MP Biomedicals, LLC, Solon, OH), alphacel (MP Biomedicals, LLC, Solon, OH), mixed vitamins (Vanderzant modification vitamin mixture for insect diet; MP Biomedicals, LLC, Solon, OH), antifungal salt solution in ethanol (methyl-*p*-hydroxybenzoate, 15:85 $g.ml^{-1}$) (ICN Biomedicals, Inc. Aurora, OH), and 25 ml of glycerol (Fisher Scientific, Pittsburgh, PA). Dog food (160 g) was mixed with water and brought to a boil. With the exception of antifungal salt, other ingredients were added to the mixture and cooked to a boil again. The antifungal salt solution was then added to the gravy and cooked for 30 min to reach the desired thickness. The 80 g of hot mixture was then added to dry whole dog food in rearing jars (85 mm diameter, 160 mm height) and cooled to room temperature (20ºC) prior to inoculation with mites. After mites were added to diets, the jars were sealed with labeled filter paper (90 mm, Fisher Scientific®, Pittsburgh, PA) in the metal lid ring. *T. putrescentiae* cultures have been stored for more than four years at 25ºC and 70% RH in darkness at our laboratory at Kansas State University and have never been exposed to extreme temperatures.

Pre- and Post-fumigation Bioassay Preparation and Mite Mortality Assessment

Mite life stages were separated into eggs and mobile stages for all SF fumigation bioassays and placed into ventilated glass vials (4.0 ml; Fisher Scientific®, Shell vial, 12 × 35 mm, Pittsburgh, PA), each including 0.2 mg of soft dog food. Each experimental vial included either a combination of twenty 7-10 d-old adults and nymphs or 10, 1-2 d-old eggs. Mites and eggs were added to each vial from the mite colony using a one-haired brush. Eggs were placed on a narrow piece of black paper (4 × 25 mm) that was covered with double-side tape. Vials were covered with fine mesh cloth (125 μm) to retain mites and to provide a fumigant gas pathway during the bioassay and humidity during the fumigation and post-fumigation periods. After fumigation, the vials were stored in fresh air within the incubator at 25ºC and 70% RH. Mortality of adults and nymphs was assessed based on lack of movement under physical stimulus after a 2-day recovery period, and unhatched eggs were considered dead four days after fumigation.

SF fumigation

SF gas from a ProFume™ gas cylinder (99.9% purity, Dow AgroScience LLC, Indianapolis, IN) was transferred to Tedlar® gas bags (CEL Scientific Crop, Santa Fe Spring, CA). The appropriate amount of SF was drawn and injected into each air-tight fumigation jar following the removal of an equal volume of air to achieve the desired SF concentration. SF at 99.9% purity was used as a standard for quantitative analysis. SF was introduced using a gas tight syringe. The start and ending concentrations of SF were measured three times by GC-MS (17A, Schimadzu Scientific Instruments, Inc., Columbia MD) right after SF injection to the fumigation jar (3.8 L, 25 cm tall by 15 cm diameter, with neck diameter of 11.4 cm) and just before the end of the fumigation period, respectively. The average of these two concentrations

was then calculated and compared to the standard SF concentration to understand the discrepancy of actual and target amounts of gas during the bioassay.

Fumigation Bioassays

Efficacy of Temperature

Vials with different life stages of mites were placed in the air tight fumigation jars. These jars were fitted out with a port in the center of the metal screw-on cap which was covered with a rubber septum through which gas was injected. Water (150 µl) was added to each jar to provide sufficient humidity to avoid desiccation of the mites. For each treatment, there were three replications of fumigation jars for a given target temperature and three sub-replicates of experimental vials in each fumigation jar. To generate the target CT product of 1400 $gh.m^{-3}$, the amount of 29.2 $g.m^{-3}$ of SF was injected to each jar, SF concentration was quantified by GC-MS and jars then were transferred in separate incubators at 25, 30, 35 and 40ºC for 48 h. Three non-fumigated control jars were stored at 25ºC for 48 h. The recovery period and mortality assessment were conducted as previously described.

Efficacy of Exposure Time

Three jars including three experimental vials of mites were fumigated with different amounts of SF ranging from 29.2 to 116.7 $g.m^{-3}$ for each desired exposure time period. To adjust the CT product to 1400 $gh.m^{-3}$ of SF, exposure times of 12, 24, 36 and 48 h were assigned to treatments. Bioassays were conducted at 40ºC and then immediately ventilated after fumigation was completed. Three non-fumigation control jars were prepared the same way and stored at 25ºC for 48 h. Recovery periods and evaluation of mortality was completed as previously described.

Time-Mortality Relationship Bioassays

CT products of SF at 300 and 1000 $gh.m^{-3}$ were targeted to determine the shortest time that could result in 50 and 99% mortality of mobile mite stages and eggs. Three prepared fumigation jars with mite vials were exposed to several doses of SF ranging from 15.5 to 105.5 $g.m^{-3}$ for 6 to 96 h at 40ºC to result in approximately 300 $gh.m^{-3}$. Two sets of non-fumigated control jars were stored at 25 and 40ºC for 96 h. A similar set of jars was prepared and fumigated from 3 to 48 h with SF doses ranging from 20.8 to 333.3 $g.m^{-3}$ to produce 1000 $gh.m^{-3}$. Control treatment jars were prepared likewise and stored at 25 and 40ºC for 48 h.

Data Analysis

Percent mite mortality exposed to combinations of SF and heat were adjusted using angular values (Mosteller and Youtz 1961) of the proportion killed and then subjected to analysis of variance (ANOVA) using the GLIMMIX procedure for each experiment. Means were separated by Tukey's HSD test when the *F*-test of the ANOVA was significant at $P < 0.05$ (SAS Institute Inc. 2010). Probit analysis was used to calculate the 50 and 99% lethal times (LT_{50} and LT_{99}) of the mortality response rate utilizing PROC PROBIT. The goodness-of-fit of the model to the data was compared using a Chi-square statistic (Robertson and Preisler 1992).

Results

Mortality of mobile stages and eggs in non-fumigated control jars, kept at 25ºC for the longest exposure time at each experiment reported below, ranged from 0 to 5.5% for all experiments and therefore treatment mortality was not corrected for control mortality. Fumigated jars in the first and second experiments (Tables 5-1 and 5-2) that targeted a CTP of 1400 $gh.m^{-3}$ had a measured average (± SE) CTP of 1250.6 ± 40.1 $gh.m^{-3}$ and 1240 ± 28.1 $gh.m^{-3}$, respectively. Fumigant jars with target CTPs of 300 and 1000 (Table 5-2) $gh.m^{-3}$ had average CTPs of 305.8 ± 8.8 $gh.m^{-3}$ and 1101.3 ± 71.1 $gh.m^{-3}$, respectively.

Mortality at 1400 $gh.m^{-3}$ for a fumigation period of 48 h was 100% for mobile stages held at either 35 or 40°C and 100% mortality of eggs was observed only at 40°C (Table 5-1). Therefore, 40ºC was the temperature used in all subsequent experiments in which holding time and/or target CTP values were varied. Mobile stages treated in jars at CTP of 1400 $gh.m^{-3}$ were all killed at every exposure time from 12 to 48 h. Eggs held at this same CTP had variable mortality with 36 and 48 h exposure resulting in 100% mortality (Table 5-2).

Exposure time also affected mortality of *T. putrescentiae* at the two lower target CT products of 300 and 1000 $gh.m^{-3}$ (Tables 5-2). Mobile stages were all killed at 36 h and longer (Table 5-2), but egg mortality averaged 98.9% at the longest hold time of 96 h (Table 5-2). All mobile stages treated with the CTP of 1000 $gh.m^{-3}$ were killed at 3 h and longer, while mortality of eggs varied between 20.0% at 3 h and 97.8% at 96 h (Table 5-2). Mortality of eggs held with no SF at 40ºC for the longest hold times in these last two experiments averaged 83.3% at 48 h, and this was significantly lower than the 95.5% mortality for those held with SF for 48 h ($F_{1, 16}$ =9.7, $P < 0.01$), while mite eggs held at 40ºC with air only for 96 h experienced 95.5% mortality, which was not different from the 98.9% killed during the same time period with SF in the jars

($F_{1, 16} = 2.6$, $P = 0.1$). Probit analyses estimated that the lethal times to kill 99% of *T. putrescentiae* eggs were 46.4 h at 1000 $gh.m^{-3}$ of SF and 87.7 h for 300 $gh.m^{-3}$ of SF (Table 5-3). Results indicate that approximately 99% of all mites (eggs and mobile stage) could be killed in a 48-h exposure to 21.5 $mg.m^{-3}$, but a longer hold time of 96 h or more was necessary at a concentration of 3.4 mgm^{-3} to achieve a similar mortality.

Discussion

The possibility of controlling all *T. putrescentiae* life stages through altering the temperature, exposure time, and SF concentration with the requirement keeping the SF CTP under the maximum EPA label rate, 1500 $gh.m^{-3}$ was evaluated in this study (Thoms et al. 2008). It has been demonstrated in a former study that *T. putrescentiae* eggs are the most tolerant life stage to control by SF at room temperature (Phillips et al. 2008). Complete mortality of eggs was not achieved even at SF concentrations that were three times greater than the EPA label rate after 48 h (Phillips et al. 2008). Results from the current study indicated that *T. putrescentiae* eggs are more difficult to control than adults and nymphs with SF as they required higher temperatures, longer exposure times and a higher gas concentration when the other two variables were held constant.

Parallel trends among life stage susceptibility to SF have been noted in several insect species for which eggs either require higher concentrations or longer exposure times to control. For instance, eggs of the beetles furniture carpet beetle, *Anthrenus flavipes* (LeConte) (Coleoptera: Dermestidae), black carpet beetle, *Attagenus unicolor* (F.) (Coleoptera: Dermestidae), *L. serricorne*, and hide beetle, *Dermestes maculatus* De Geer (Coleoptera: Dermestidae), were controlled at 7 to 30 times higher SF concentrations for 22 h than adults and larvae at 26.5ºC (Su and Scheffrahn 1990). Adults of psocids, *Lepinotus reticulatus* Enderlein (Psocoptera: Trogiidae) and *Lepinotus decolor* Pearman (Psocoptera: Trogiidae), were killed at one third and half of the SF concentration ($g.m^{-3}$) when compared to the required concentration to control eggs of the same species after 48 h at 27.5ºC (Athanassiou et al. 2012). The Japanese pine sawyer beetle, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae), egg mortality

was 100% at 2400 gh.m^{-3} of SF at 25°C for 24 h, and this dose was five-fold greater than the dose required for either larvae or pupae of *M. alternatus* (Soma et al. 1997). Baltaci et al. (2009) reported 208.8 gh.m^{-3} of SF at 15°C was adequate to kill all larvae and pupae of the tobacco moth, *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae), but an SF CTP that was five times greater at 25°C was necessary to control eggs. The poor ovicidal activity with SF has been attributed to inhibition of gas penetration by the proteinaceous chorion and embryonic membranes (Outram 1967).

Phillips et al. (2012) stated that the major factors affecting proper fumigation include target pest species, the most tolerant life stage to that particular fumigant, fumigant concentration, length of exposure period, and the temperature of the fumigated environment. Elevated temperature has been suggested to increase SF toxicity through greater fumigant uptake and accelerated metabolism (Reichmuth and Klementz 2008). In addition, greater temperatures have been shown to increase the mortality of eggs of other stored product pests, including wood borers and bed bugs (Reichmuth et al. 2003, Barak et al. 2006, Phillips et al. 2014). Mueller (2007) reported that 20 g.m^{-3} of SF at 20ºC for 64 h did not control the mobile stages of *T. putrescentiae* and low mite mortality (<50%) was obtained. Results from the current study indicated that all life stages of *T. putrescentiae* could be controlled at 40ºC, which is in agreement with Thoms et al. (2008), who reported that all life stages of several stored-product pests can be controlled by SF with temperatures ranging from 20 to 40°C without required dosages that are greater than 1500 gh.m^{-3}. Moreover, all life stages of the sawtoothed grain beetle, *O. surinamensis*, lesser grain borer, *Rhyzopertha dominica* (F.), rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), red flour beetle, *T. castaneum* and Khapra beetle, *Trogoderma granarium* Everst (Coleoptera: Dermestidae), pests that are known to infest bagged

rice and wheat stacks were controlled by SF at about 960 gh.m^{-3} after 24 h at 32 ± 7°C (Rajendran et al. 2008). Flour mill fumigation in Germany indicated that all life stages of the stored product insects, *E kuehniella, Plodia interpunctella* (Hubner) (Lepidoptera:Pyralidae)*, T. castaneum, T. molitor* and *O. surinamensis* mortality were greater than 99% at 2255 gh.m^{-3} of SF and 40 °C after 48 h (Reichmuth et al. 2003). Infestation of the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), decreased by more than 96% at 3 months post-fumigation with SF at 30°C (Small 2007).

Previous studies indicated that fumigation at greater temperatures reduce the concentration of SF. Phillips et al. (2014) studied bed bugs, *Cimex lectularius* L. (Hemiptera: Cimicidae), and showed that the SF needed for achieving LAD_{99} (Lethal accumulated dose) of eggs at 15ºC could be reduced by 50% to achieve the same mortality at 25ºC. A 10°C increase in temperature reduced the required SF CTP (gh.m^{-3}) by one third to control adults of *E. kuehniella* (Bell et al. 1998, Reichmuth et al. 1999, Bell et al. 2003). Bonifácio et al. (2014) reported that pinewood nematodes were completely controlled with half as much SF (2141 gh.m^{-3}) that was required at 30ºC after 24 h when compared to the amount (4407 gh.m^{-3}) needed at 15ºC. Reichmuth et al. (1998) showed that a small increase in temperature can considerably affect mortality of stored-product moth eggs with lower concentrations of SF. Results from the current study indicated that increased temperature can offer complete control of ham mites at a lesser CTP of SF as compared to the very high CTPs tested by Phillips et al. (2008) at 23ºC. However, comparison of egg tolerance to SF indicates that ham mite eggs are among the most tolerant egg stages of various stored-product pests. Bell et al. (2003) reported that an SF CTP of 1,000 gh.m^{-3} was needed to achieve 100% mortality of *L. bostrychophila* eggs after 48 h exposure at 25ºC.

Another study indicates that approximately 1000 gh.m^{-3} of SF at 15ºC caused 100% egg mortality in *E. elutella* after 48 h (Baltaci et al. 2009).

Increasing exposure times of *T. putrescentiae* life stages to SF, while staying at the same approximate CTP values (decreased gas concertation at longer hold times), can improve control, which has also been the case for other pest species. For instance, when SF fumigation time was prolonged from one day to two days, greater mortality of several postharvest pest species was obtained (Reichmuth et al. 1998, Schneider and Hartsell 1999, Williams and Schneider 1999, Reichmuth and Klementz 2008). The current study indicates that longer exposure times for CTPs of either 300 or 1000 gh.m^{-3} of SF caused greater mortality in all stages of *T. putrescentiae* at 40ºC. Egg mortality was moderate during the first 12 h of fumigation under heat, then increased markedly from 12 to 36 h, with100% mortality of eggs achieved at 96 h. A tolerant life stage like the egg may develop to the next susceptible stage under a relatively short fumigation, but a longer fumigation or a second fumigation would be needed to kill all stages and offer a complete infestation extermination (Reichmuth and Klementz 2008).

No single control method can likely replace methyl bromide for post-harvest pest control (Field and white 2002). Phosphine is very effective in controlling ham mites within the label rate (Schilling et al. 2010) but it causes metallic corrosion to electrical devices and thus is very impractical (Phillips et al. 2012). Bell (2003) conducted SF fumigations at 50ºC and CTP of over 22,000 gh.m^{-3} demonstrated no sign of corrosion on computers and copper pieces exposed to the gas. Additionally, SF is a gas under every applied condition since it has a boiling point of -55.4ºC and very good penetration capacity as compared to methyl bromide (Baltaci et al. 2009, Zettler and Arthur 2000). Combining SF with controlled atmospheres such as high CO_2 improved pest control when compared to SF alone (Scheffrahn et al. 1995). Fields

and White (2002) concluded that some of the best alternatives to methyl bromide fumigation include extreme temperature treatment and also fumigation with SF. The current project suggests that the combination of SF and heat could be a useful methyl bromide alternative as part of an integrated pest management program in dry-cured ham facilities. Commercial application of SF with heat in ham facilities will require additional research on fumigation methods, food safety and food quality.

References

Athanassiou, C. G., T. W. Phillips, M. J. Aikins, M. M. Hasan, and J. E. Throne. 2012. Effectiveness of sulfuryl fluoride for control of different life stages of stored-product Psocids (Psocoptera) 105: 282–287.

Baltaci, D., D. Klementz, B. Gerowitt, M. J. Drinkall, and C. Reichmuth. **2009**. Lethal effects of sulfuryl fluoride on eggs of different ages and other life stages of the warehouse moth *Ephestia elutella* (Hübner). J. Stored Prod. Res. 45: 19–23.

Barak, A. V, Y. Wang, G. Zhan, Y. Wu, L. Xu, and Q. Huang. **2006**. Sulfuryl fluoride as a quarantine treatment for *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in regulated wood packing material. J. Econ. Entomol. 99: 1628–1635.

Bell, C. H., and M. J. Drinkall. **2000**. Sulfuryl fluoride to replace methyl bromide use in the flour milling industry. *In* Proc. Annu. Int. Res. Conf. Methyl Bromide Altern. Emiss. Reduct.

Bell, C. H., and N. Savvidou. **1998**. The toxicity of Vikane (sulfuryl fluoride) to age groups of eggs of the Mediterranean flour moth (*Ephestia kuehniella*). J. Stored Prod. Res. 35: 233–247.

Bell, C. H., N. Savvidou, and T. J. Wontner Smith. **1999**. The toxicity of sulfuryl fluoride (Vikane®) to eggs of insect pests of flour mills. Proc. 7th Int. Work. Conferece Stored-Product Prot. 1: 345-350. October 14–19. Chengdu, China

Bell, C. H., T. J. Wontner-Smith, N. Savvidou, P. F. Credland, D. M. Armitage, P. M. Cogan, and E. Highley. **2003**. Some properties of sulphuryl fluoride in relation to its use

as a fumigant in the cereals industry, *In* Adv. Stored Prod. Prot. Proc. 8th Int. Work. Conf. Stored Prod. Prot. York, UK, 22-26 July, 2002. CABI Publishing. pp. 910–915

Boczek, J. 1991. Mite pests in stored food, *In* Ecology and Management of Food-Industry Pests, ed. Gorham JR. AOAC Press, Arlington, VA. pp. 57-79.

Boina, D. R., B. Subramanyam, and S. Alavi. **2008**. Dynamic model for predicting survival of mature larvae of *Tribolium confusum* during facility heat treatments. J. Econ. Entomol. 101: 989–997.

Bonifácio, L. F., E. Sousa, P. Naves, M. L. Inácio, J. Henriques, M. Mota, P. Barbosa, M. J. Drinkall, and S. Buckley. **2014**. Efficacy of sulfuryl fluoride against the pinewood nematode, *Bursaphelenchus xylophilus* (Nematoda: Aphelenchidae), in *Pinus pinaster* boards. Pest Manag. Sci. 70: 6–13.

Emekci M. 2010. Section: fumigation, modified atmospheres and hermetic storage. *In* 10th Int. Work. Conf. Stored Prod. Prot. Lisbon, Portugal.

Fields, P. G. 1992. The control of stored-product insects and mites with extreme temperatures. J. Stored Prod. Res. 28: 89–118.

Fields, P. G., and N. D. G. White. **2002**. Alternatives to methyl bromide treatments for stored-product andquarantine insects. Annu. Rev. Entomol. 47: 331–359.

Fields P., Subramanyam B., Hulasare R. 2012. Extreme temperatures, In Hagstrum et al (eds), Stored-product protection. S156. Kansas State University Research and Extension publication, Manhattan, KS, USA. pp. 179–190.

Marriott, N. G., and M. W. Schilling. **2004**. Dry cured pork research review white paper. National country ham association, Inc. National country ham association, *In* Annu. Meet. pp. 1–62.

Mosteller, F., and C. Youtz. **1961**. Tables of the freeman-Tukey transformations for the binomial and poisson distributions . Biometrika . 48 : 433–440.

Mueller, D. K., P. J. Kelley, and A. R. VanRyckeghem. **2006**. Mold mites *Tyrophagus putrescentiae* (Shrank) in stored products, p. 1122. *In* 9th Int. Work. Conf. Stored Prod. Prot.

Navarro, S. 2006. New global challenges to the use of gaseous treatments in stored products. ed. Lorini, I.; Bacaltchuk, B.; Beckel, H. pp. 495–509.

Outram, I. 1967. Factors affecting the resistance of insect eggs to sulphuryl fluoride—II: The distribution of sulfuryl-35S fluoride in insect eggs after fumigation. J. Stored Prod. Res. 3: 353–358.

Phillips, T. W., M. J. Aikins, E. Thoms, J. Demark, and C. Wang. **2014**. Fumigation of bed bugs (Hemiptera: Cimicidae): Effective application rates for sulfuryl fluoride. J. Econ. Entomol. 107: 1582–1589.

Phillips, T. W., M. M. Hasan, M. J. Aikens, and M. W. Schilling. **2008**. Efficacy of sulfuryl fluoride to control ham mites and red-legged ham beetles. *In* Annu. Int. Res. Conf. Methyl Bromide Altern. Emiss. Reduction, Orlando, FL, Novemb.

Phillips, T. W., E. M. Thoms, J. DeMark, and S. Walse. **2012**. Fumigation. In Hagstrum et al (eds), Stored-product protection. S156. Kansas State University Research and Extension publication, Manhattan, KS, USA. pp. 157–179

Rajendran, S., V., K. Lalith, and V. Sriranjini. **2008**. Fumigation of grain stacks with sulfuryl fluoride. Int. pest Control. 50: 192–195.

Reichmuth, C., and D. Klementz. **2008**. How to overcome the egg-weakness of sulfuryl fluoride–combination of control methods. *In* Proc. Annu. Int. Res. Conf. Methyl Bromide Altern. Emiss. Reductions. Citeseer.

Reichmuth, C., W. Rassmann, G. Binker, G. Fröba, M. J. Drinkall, P. F. Credland, D. M. Armitage, C. H. Bell, P. M. Cogan, and E. Highley. **2003**. Disinfestation of rust-red flour beetle (*Tribolium castaneum*), saw-toothed grain beetle (*Oryzaephilus surinamensis*), yellow meal worm (*Tenebrio molitor*), Mediterranean flour moth (*Ephestia kuehniella*) and Indian meal moth (*Plodia interpunctella*) with sulfuryl fluoride, *In* Adv. Stored Prod. Prot. Proc. 8th Int. Work. Conf. Stored Prod. Prot. York, UK, 22-26 July, 2002. CABI Publishing. pp. 736–738.

Reichmuth, C., B. Schneider, and M. Drinkall. **1998**. Sulfuryl fluoride against eggs of different age of the Indian meal moth *Plodia interpunctella* (Hubner) and the Mediterranean flour moth *Ephestia kuehniella* Zeller. Proc. 7th Int. Work. Conf. Stored-product Prot. 1: 416–422.

Rentfrow, G., D. J. Hanson, M. W. Schilling, and W. B. Mikel. **2008**. The use of methyl bromide to control insects in country hams in the Southeastern United States. Ext. Publ. Kentucky Extension/National Ctry. Ham Assoc. ASC-171. 1–2.

Robertson, J. L., and H. K. Preisler. **1992**. Pesticide bioassays with arthropodsCRC Press, Boca Raton, FL.

SAS Online Document. 2010. PROC User's manual, version 11th ed. SAS Institute. Cary, NC.

Scheffrahn, R. H., G. S. Wheeler, and S. Nan-Yao. **1995**. Synergism of methyl bromide and sulfuryl fluoride toxicity against termites (Isoptera: Kalotermitidae, Rhinotermitidae) by admixture with carbon dioxide. J. Econ. Entomol. 88: 649–653.

Schilling, M.W., Phillips, T. W., Aikins, J. M., Hasan, M. M., Sekhon, R. K., Mikel, W. B. 2010. Research update: Evaluating potential methyl bromide alternatives for their efficacy against pest infestations common to dry cured ham. National Country Ham Association Annual Meeting. Asheville, NC.

Schneider, B. M., and P. L. Hartsell. **1998**. Control of stored product pests with Vikane gas fumigant (sulfuryl fluoride). Mortality. 1: 100.

Small, G. 2009. Evaluation of the impact of sulfuryl fluoride fumigation and heat treatment on stored-product insect populations in UK flour mills. Int. Pest Control. 51: 43–46.

Small, G. J. 2007. A comparison between the impact of sulfuryl fluoride and methyl bromide fumigations on stored-product insect populations in UK flour mills. J. Stored Prod. Res. 43: 410–416.

Soma, Y., M. Mizobuchi, T. Oogita, T. Misumi, H. Kishino, T. Akagawa, and F. Kawakami. **1997**. Susceptibility of forest insect pests to sulfuryl fluoride, 3: Susceptibility to sulfuryl fluoride at 25degree centigrade. Res. Bull. Plant Prot. Serv.

Su, N.-Y., and R. H. Scheffrahn. **1990**. Efficacy of Sulfuryl fluoride against four beetle pests of

museums (Coleoptera: Dermestidae, Anobiidae). J. Econ. Entomol. 83: 879–882.

Thind, B. B. 2005. A new versatile and robust mite trap for detection and monitoring of storage mites in the cereal and allied industries. Exp. Appl. Acarol. 35: 1–15.

Thind, B. B., and P. G. Clarke. **2001**. The occurrence of mites in cereal-based foods destined for human consumption and possible consequences of infestation. Exp. Appl. Acarol. 25: 203–215.

Thoms, E., J. Busacca, and S. Prabhakaran. **2008**. Commercializing a new fumigant: The ProFume® success story. *In* Proc. 8th Int. Conf. Control. Atmos. Fumigation Stored Prod. pp. 21–26

United States Environmental Protection Agency. **2016**. Phaseout of class I ozone-depleting substances. Http://Www.Epa.Gov/.

Williams, R. E., and B. M. Schneider. **1999**. Enhanced fumigation efficacy as a methyl bromide alternative: Case studies with sulfuryl fluoride. Proc.Annu.Int.Res.Co. Methyl Bromide Altern. Emiss. Reductions. 63.

Žďárková, E. 1993. The effects of physical factors on survival of stored food mites. Exp. Appl. Acarol. 17: 197.

Zettler, J. L., and F. H. Arthur. **2000**. Chemical control of stored product insects with fumigants and residual treatments. Crop Prot. 19: 577–582.

Zhao, Y., S. Abbar, B. Amoah, T. W. Phillips, and M. W. Schilling. **2016**. Controlling pests in dry-cured ham: A review. Meat Sci. 111: 183–191.

Table 5-1 Mortality of *T. putrescentiae* exposed to 1400 gh.m^{-3} of SF after 48 h at different temperatures.

Life Stage	Temperature °C	% Mean Mortality (SE)[a]	ANOVA Results	
			$F_{(3, 8)}$	*P*
Mobile Stages	25	87.2 (2.2) b	24.2	< 0.01
	30	88.3 (2.8) b		
	35	100.0 (0.0) a		
	40	100.0 (0.0) a		
Eggs	25	11.1 (4.2) b	104.8	< 0.01
	30	12.2 (4.3) b		
	35	26.7 (4.1) b		
	40	100.0 (0.0) a		

[a] Mean values within a given experiment that have the same lower case letter are not significantly different ($P \geq 0.05$; HSD)

Table 5-2 Mortality of *T. putrescentiae* exposed to different SF CTPs at 40ºC for different exposure times.

Target SF CTP ($gh.m^{-3}$)	Life stage	Time (h)	% Mean mortality (SE)[a]	ANOVA Results
1400	**Eggs**	12	58.6 (4.3) b	$F_{3, 8} = 96.7$, $P < 0.01$
		24	97.8 (1.5) a	
		36	100.0 (0.0) a	
		48	100.0 (0.0) a	
300	**Mobile Stages**	6	66.7 (0.7) c	$F_{6, 14} = 13.6$, $P < 0.01$
		12	82.8 (0.9) ab	
		24	93.9 (0.4) ab	
		36	100.0 (0.0) a	
		48	100.0 (0.0) a	
		72	100.0 (0.0) a	
		96	100.0 (0.0) a	
300	**Eggs**	6	2.2 (0.2) d	$F_{6, 14} = 132.7$, $P < 0.01$
		12	8.9 (0.3) d	
		24	43.3 (0.5) c	
		36	57.8 (0.3) bc	
		48	63.3 (0.4) b	
		72	75.6 (0.1) b	
		96	98.9 (0.1) a	
1000	**Eggs**	3	20.0 (0.3) e	$F_{6, 14} = 19.2$, $P \leq 0.05$
		6	43.3 (0.6) de	
		9	56.7 (0.7) cd	
		12	71.1 (1.1) bcd	
		24	84.4 (0.3) ac	
		36	98.9 (0.1) a	
		48	97.8 (0.2) ab	

[a] Mean values within a given experiment that have the same lower case letter are not significantly different ($P \geq 0.05$; HSD)

Table 5-3 Probit analysis of mortality for *T. putrescentiae* eggs fumigated with SF at 40ºC.

Life Stage	Target SF CTP ($gh.m^{-3}$)	LT_{50} (95% CL)	LT_{99} (95% CL)	Slope (SE)	Intercept (SE)	χ^2	df	*P*
Eggs	300	34.89 (32.37-37.39)	87.70 (71.43-125.71)	4.37 (0.68)	-1.40 (1.90)	43.48	60	0.95
Eggs	1000	8.78 (7.42-9.98)	46.42 (38.22-60.93)	2.62 (0.25)	-0.11 (0.63)	70.72	60	0.16