

Alternative integrated pest management methods for controlling *Tyrophagus putrescentiae*
(Schrank) (Sarcoptiformes: Acaridae).

by

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Abstract

Tyrophagus putrescentiae (Schrank) (Acaridae), commonly known as the mold mite, cheese mite and ham mite, is one of the most important pests of dry-cured hams. Methyl bromide is the most effective fumigant that has been used to control ham mites. Methyl bromide is now banned for most food uses and is being phased out of use in many countries in accord with the Montreal Protocol on substances that deplete the ozone layer of the earth's atmosphere. This work is part of a long-term effort to find alternatives to methyl bromide for use in controlling ham mites. In an integrated pest management program, different tools play a role in the successful control of insect pests. Hence, effective, feasible and economically friendly integrated pest management (IPM) tools are needed to help in the control of these ham mites in the dry-cured ham industry. Work for this dissertation is divided into four independent sections by chapter, as described below.

Plant essential oils and food-safe compounds were evaluated as repellents for their effectiveness at controlling mites. Experiments to assess the repellency to orientation, oviposition, and population growth of mites on 5 mm³ and 25 mm³ pieces of aged country hams were conducted. Test compounds at different concentrations were dissolved in required solvents and compared to the solvent control. Results indicated that nootkatone and a blend of C8, C9 and C10 straight-chain hydrocarbons had repellency indices of (RI) of 85.6% and 82.3%. Geraniol had the highest RI of 96.3% at 0.04 mg/cm². Ham pieces dipped in C8910 and nootkatone at 150 ppm each had RIs of 89.3% and 82.8%, respectively. In general, as the concentrations of test compounds increased, the numbers of eggs that were laid on these treated ham cubes decreased after the 48h exposure time.

In the second study, about fifteen commercially available acaricides were screened as surface applications, and four chemicals were effective against mites. The persistence of these four chemicals over time was further assessed over eight-week periods by applying the recommended rate to three different surfaces: metal, concrete and wood. Results indicated that acequinocyl and amitraz were very persistent and effective at killing mites throughout the experiment period even after the sixth to eighth week aging of the sprayed surfaces, hence suggesting good efficacy for commercial use.

Traditionally, the use of residual pesticides and fumigants have been the primary way to control stored product pests, including ham mites. Since the use of these products are being minimized to embrace more environmentally friendly and humanly safe alternatives, the objective of this third experiment was to evaluate different physical barriers to exclude ham mites in a simulated ham-aging facility. The physical treatments included both liquid and sticky barriers. The liquid treatments were water, soapy water, vegetable oil and mineral oil whereas the dry sticky treatment were petroleum jelly and a sticky coating used in insect traps. These experiments indicated that physical barrier treatments can significantly reduce the number of ham mites that may walk up from the floor to infest dry cured hams while they were hanging on racks above the floor. However, barrier methods used for exclusion alone cannot eradicate ham mites in an aging room. Hence, it can certainly be used in conjunction with other pest management tools for successful protection of dry-cured hams.

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Dedication

This Dissertation is dedicated to the Lord God Almighty and my late mom and sister; Mrs. Agnes Fobi Manu and Mrs. Vivian Manu-Konadu. I wish they were here to see the person I have become and be happy for my achievements.

Chapter 1 - Introduction and Literature Review

History and Production of Dry-cured hams

People have been raising animals for sustenance for millenia. Prior to the present era of technology, preservation techniques relied on salting, which is linked to the ancient Egyptians for using salt to preserve the dead and food for the living (Tannahill 1973, Voltz and Harwell 1999, Rentfrow et al. 2012). Dry curing, with or without cold smoking, was a popular technique to facilitate a long-term storage of the animals' carcasses. Dry curing utilized the natural environment as well as salt (Mohan and Sevcech 2020). Currently, salting is no longer required as a preservation technique for meats due to refrigeration technology and the incorporation of additives such as nitrates and ascorbic acid to preserve meat products (Toldrá et al. 1997).

Dry-curing of hams originated in Asia and moved to Europe, where many tribes devised their own methods of curing meat (Mohan and Sevcech 2020). Pigs were reportedly first introduced into the United States, Florida by Hernando de Soto; where pigs learned to thrive in their new environment and became a prominent food source for early colonists. However, the term country ham was established in the 1940's (Rentfrow et al. 2012). Some examples of dry-cured ham include Chinese Jinhua and Yunnan hams, Italian Prosciutto, Spanish Serrano and Iberian hams, and German Black Forest hams. (Rentfrow and Suman 2014). The term country ham was established in the 1940s to describe a type of dry-cured ham and these types of hams are currently primarily produced in Kentucky, Tennessee, North Carolina, and Virginia (Rentfrow et al. 2012). The National Country Ham Association (NCHA) had 20 members in 2005, but has since grown, producing 3.4 million hams worth more than 340 million dollars in

retail sales (Ramos 2006). However, their numbers have gotten smaller over the last twenty years.

Different countries have their own style of dry-cured ham. Some current examples of dry-cured ham include Chinese Jinhua and Yunnan hams, Italian Prosciutto, Spanish Serrano and Iberian hams, and German Black Forest hams (Rentfrow and Suman 2014). The breed, feed, weight and age of pig impact the production process of the dry-cured ham (Damodaran and Fennema 2008, Toldrá 2002). High-quality dry-cured hams with a production time of more than a year have particular organoleptic qualities, such as a rich, distinct, and recognized flavor, as well as a color range from rosy to maroon, or brown-red marbled with white fat (Toldrá 2002, Petrova et al. 2015). The sensory, physical–chemical, aromatic, morphological, and textural features of dry-cured ham vary greatly depending on technological process variations from producer to producer (Toldrá and Flores 1998, Virgili et al. 2007, Benedini et al. 2012 and Laureati et al. 2014). American dry-cured ham is the legal definition of the hind leg of the pork carcass that has been cured with dry salt and lost at least 18 % of its original weight, but contains at least 4 % salt owing to dehydration throughout the curing and maturing process (USDA 1999).

Hams are cured at an average daily temperature of 2 to 4 °C, and the amount of time it takes to cure is determined by the size and weight of the fresh ham. (Toldrá et al. 1997, USEPA 2010). Hams can be cured in either natural or artificial environments, using one of two curing methods: bagged or salt box (Rentfrow et al. 2012). It is recommended that every hole in the curing building be sealed, as this will keep the ham free of unwanted odors since hams have a tendency to acquire undesirable smells from their surroundings (Rentfrow and Suma 2014, Mohan and Sevcech 2020).

The usual dry-cured ham production method includes salting, salt equalization, post-salting (resting), and drying–ripening (Zhao et al. 2016). The ham is cured with salts, smoked occasionally, and then matured for several months (Marriott and Ockerman 2004). A standard dry-cured recipe uses 4 kg of salt and 1 kg of white or brown sugar, along with black and/or red pepper or paprika. A cure mixture weighing about 10 kg will cure 100 kg of green ham. (Marriot and Schilling 2004, USEPA 2010). Sodium nitrate is commonly used but are not required for this process (Rentfrow et al. 2012).

Salt inhibits the multiplication of spoilage bacteria by lowering water activity and increasing myofibrillar protein solubility whereas nitrate is broken down into nitrite and then oxide, which gives the ham its pink color and flavor (Toldrá et al. 1997, Armenteros et al. 2012). The key processes that contribute to the ultimate quality of dry-cured ham are proteolysis and lipolysis (Petrova et al. 2017). Natural moisture in the ham dissolves the ingredients, which eventually diffuse into the ham. The USDA recommends a minimum of four days of curing per kilogram of ham. Pathogen growth in ham could occur if it is cured for less than the USDA-recommended 40 days or 2 days per pound (Rentfrow et al. 2012, USDA 9 CFR 318.2011, Mohan and Sevcech 2020). During this process, the relative humidity is kept below 75 % to prevent microbial development and the formation of undesirable flavors (Marriott and Ockerman 2004).

Cure equalization is accomplished by storing hams at 10-13 °C for around 2 weeks after the curing processes. During the curing and equalization processes, the hams shrink by 8–10% (Marriott and Schilling 2004, Graham et al. 2012). As a last stage, hams are placed in ventilated rooms and subjected to time-temperature-relative humidity cycles which is referred to as the aging or ripening period (Graham et al. 2012). The optimal temperature and relative humidity

during aging ranges from 16–28°C and 65– 80% in the United States (Toldrá 2010, Rentfrow et al. 2012). Hams lose 8–12 % of their original weight during the aging process (Graham et al. 1998). Depending on the time of maturing, the ham develops a darker color, firmer texture, and more desirable flavor (Toldrá et al. 1997). Flores and Toldrá (1993) describe the end result as a shelf-stable, dry-cured ham with a distinct flavor. Most country ham manufacturers in the United States age hams for 3–6 months, while others age hams for further 18 to 36 months to enhance the flavor (Rentfrow et al. 2012).

Pests of Dry Cured Ham

Dry cured hams are subjected to complex arthropod pest infestations because they have high protein and moisture content, providing a high-quality food supply for pests like ham mites (Jimenez-Colmenero et al. 2010, Rentfrow et al. 2012). Dry-cured hams that are stored under ambient conditions are especially prone to mites, beetles, and maggots when the climatic circumstances are conducive for their survival and growth. Eventhough pest infestation occur during aging the high value of dried hams in terms of production and consumer expectations remains (Riudavets 2004, Rentfrow and Summax 2014). *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae), *Dermestes lardarius* L. (Coleoptera: Dermestidae), *Necrobia rufipes* (F.) (Coleoptera: Cleridae), and *Piophilila casei* (L.) (Diptera: Piophilidae) are the common pests of dry-cured ham (Graham et al. 2012, Rentfrow and Suma 2014, Zhao et al. 2016).

When ham is aged for less than three months, there is minimal or no infestation. However, to achieve the appropriate flavor and product quality for a specialized high-dollar market, it is important to consider aging for more than six months (Zhao at al. 2016). When ham is matured for more than five months, the risk of mite infestation rises but aging hams for less

than five months does not guarantee that there will be no infestation issues (Rentfrow et al. 2006).

Ham mites, *T. putrescentiae*, generally infest the surfaces of the dry cured hams although mites might permeate the product at higher population levels, resulting in a higher economic loss (Zdárková 1998). Large numbers of mites can generate mite dust, which is a mixture of mites cast skin, exuvia, dead mites and live mites, around the infested food product and the white specs on the infested surface may appear to move when observed with the naked eye (Zhao et al. 2016). More information on this species is provided in a subsequent section.

Red-legged ham beetle, *Necrobia rufipes*, is a worldwide pest that infests high-protein preserved goods such as copra (coconut meat), cheese, dried fish, dried meat and ham, causing severe damage (Simmons and Ellington 1925). Adults have pale yellowish to reddish legs and dark blue to metallic green bodies, and are around 3.5 to 7 mm long (Lambkin and Khatoon 1990, Odeyemi 1997). The life cycle can last anywhere from one to five months or longer, depending on the type of food available and environmental conditions. A temperature range of 30–34°C and 50% relative humidity or above are excellent development conditions for *N. rufipes* (Haines and Rees 1989). Adult red-legged beetles may live for more than 14 months in warm (30 to 33°C) and humid circumstances, and females can lay up to 28 eggs per day and 3000 eggs in one life cycle (Roesli and Subramanyam 2002).

The larder beetle, *D. lardarius*, is a worldwide pest of cured meat, cheeses, dried fish, museum items, and pet food (Haines and Rees 1989). Adults are dark brown with a yellowish line across the front half of the forewings and measure 7–9 mm in length. During the summer months, females lay over 100 eggs, and the life cycle is completed in less than two months under optimal conditions (Jacobs 2013).

Cheese skippers, *Piophilidae casei*, sometimes known as cheese flies or ham skippers, feed on overripe or moldy cheese as well as salty meats such as dry-cured ham and beef (Lewis and Kaufman 2010). Females lay 140–500 eggs on meat and cheese in a single life cycle. Adults survive for three to seven days and go through a whole life cycle that lasts anywhere from 12 to 30 days (Lewis and Kaufman 2010). The burrowing larva is the most damaging stage since it penetrates and eats deeply into the food products (White et al. 2006).

Ham Mites as Key Pests of Dry-Cured Hams

T. putrescentiae are cosmopolitan acari that are found all over the world (Kheradmand et al. 2007). It is sometimes classified as a part of the house dust mite complex, and it has been linked to allergic reactions in humans (Jeong et al. 2005, Wakefield 2006). The ham mite was first described by Schrank in 1781 and named *Acarus putrescentiae* (Klimov and Oconnor 2010). It is an astigmatid mite in the family Acaridae. *Tyrophagus putrescentiae* has several synonyms, including *Tyroglyphus lintneri* Osborn (1893) and *Tyroglyphus castellanii* Hirst (1912), but a comprehensive survey revealed that the name *Tyrophagus putrescentiae* has been used in the majority of papers published in the last 20 years (Klimov and Oconnor 2010, Farrell and Nayak 2012). Stigmata and external breathing apertures are absent in astigmatid mites (Olsen 1995). In addition to ham mites, some other common names for this species of mite are mold mite, copra mite, cheese mite, cereal mite, fodder mite, and mill mite (Rentfrow et al. 2008, Farrell and Nayak 2012).

Ham mites survive well under environmental conditions of temperature of 23.2 ± 2.1 °C and relative humidity (RH) of 71 ± 5.6 % (Adler 2004, Riudavets 2004). Mites survive in moist, moldy and tropical environments. Unless assisted vision is used, they are not apparent to the naked eye. They can go undetected until a commodity is extensively infested. At high densities

they can look like a moving dusty surface on a commodity and a layer of fine pale brownish material known as ‘mite dust’ which comprises both live and dead mites, as well as, their cast skin and exuvia, can accumulate. Ham mites are often discovered at the bottom of storage bags of contaminated commodities (Mueller et al. 2006).

Biology, Life Cycle and Host Range of Ham Mites

The ham mite has a small body (0.53mm) and eight walking legs. Some of them have complete body segment however others do not have body segmentation (Farrell 2018). Ham mites are synanthropic and feed on high fat, high protein and high moisture foods like bacon, cheese and sausages (Kheradmand et al. 2007, Qu et al. 2015). Other commodities that they can infest include cereals, include cereals, peanuts, medicinal herbs, copra, dried eggs, dry milk, sunflower seed, tobacco, flour, mushrooms, mold spores, and yeast hyphae (Rodriguez and Rodriguez 1987, Duck et al. 2001, Chambers 2002).

Adult ham mites mate quickly after eclosion, with copulation lasting 1.5 hours. When females have a plentiful and consistent food supply, they lay eggs. As long as there is a food source and good climatic conditions, a single male mite can fertilize roughly 450 females, and females can lay eggs within 24 hours of mating (Gorham 1991). There have been varied estimates of how many eggs a female can lay in her lifetime: 80 eggs (Bahrami et al. 2007) and 500 eggs over 45 days (Duck et al. 2001); when mated at least twice a month, a single female can lay roughly 500 eggs (Duck et al. 2001); 60 eggs per day, but on average, only lays 4 eggs per day, and about 70% of her eggs are laid in the first three weeks of her adult life (Gorham 1991).

According to Farrell (2018), temperature and relative humidity in the micro-environment and food sources are the major contributors that influence the growth and development of ham

mites. The developmental stages of the mold mite comprise of an egg, larva and nymph with typical developmental times of 2, 3, 5 days, respectively and egg to adult can take 10 (Bahramni et al. 2007) or 16 days (Duek et al. 2001). At an optimum temperature of 32.2 °C and relative humidity of 98 %. Barker (1967) observed rapid growth of eggs and immature mites and they developed fully in about 80 hours.

Economic Importance of Ham Mites

T. putrescentiae causes serious economic losses to stored products by lowering nutritional content, germination capacity also causes economic losses as a contamination and negative impacts on customers leading to product returns and decreased sales (Krantz 1955). Additionally, the mite produces acute allergic reactions in farm workers and those working in the food business who handle extensively infested stored goods (Hughes 1976). When food is contaminated with ham mites, it can cause enteritis (Hughes 1976) and systemic anaphylaxis (Matsumoto et al. 1996). In grain that is kept in warm, moist conditions for storage, the mite also serves as a carrier of bacteria and toxic fungi like *Aspergillus* spp (Franzolin et al. 1999). Customers who store food products—including pet food—in their homes run the risk of developing a mite infestation because some storage mites can be found in house dust. Storage mites were discovered in two out of ten different brands of sealed commercial dog foods when the foods were kept at the appropriate temperatures and humidity levels (Brazis et al. 2008).

Control Measures Employed for Ham Mites

Methyl Bromide and Other Synthetic Fumigants

A fumigant is defined as a harmful gas that is capable of penetrating porous surfaces or enclosed spaces under a variety of temperatures and pressures in order to eradicate all phases of a pest organism's development (Thoms and Phillips 2004). The target pest, type of commodity

fumigated, most tolerant life stage to that particular fumigant, fumigant concentration, duration of exposure period, and temperature of the treated environment are all key elements determining proper application of a fumigant (Phillips et al. 2012).

Methyl bromide (CH_3Br) has been used commercially for over 40-60 years to control stored product pests (Fields and White 2002). It is a colorless, odorless, noncorrosive, broad-spectrum, and nonflammable fumigant which can spread evenly throughout the fumigated area and commodities, ensuring pest disinfestation during exposure while leaving little or no residues on products (Bond 1984, Fields and White 2002). It was the fumigant used to control stored product insects associated with the dry-cured ham such as cheese skippers, dermestid beetles, ham beetles as well as other stored product pests like mites, rodents, microflora, and nematodes (Fields and White 2002, Schilling et al. 2004, EPA 2006). Any mite infestation throughout the aging period of dry cured ham is considered adulterated in the United States, and producers are required to eliminate mites before sale (USDA 9 CFR 301 2011, USDA 9 CFR 416 2011).

Methyl bromide has been used in controlling ham mites in an aging room over the years (Marriott and Schilling 2004). According to the Montreal Protocol, which was signed by 196 countries and was created to safeguard the world from the harmful effects of ozone-depleting compounds. Methyl bromide was to be phased out on a timetable for both developed and developing countries (US EPA 2010). It was an extremely effective fumigant. However, it posed a threat to the environment by depleting stratospheric ozone layer of the earth's atmosphere (US EPA 2006). Only critical use exemptions (CUE) for methyl bromide were nominated and allowed under the Montreal Protocol and the United States Clean Air Act to give industries time to study methyl bromide alternatives and transition from using methyl bromide to alternatives tools. Postharvest uses accounted for less than 10 % of the entire allowance, with mills and

processors receiving 71–92 % whereas 90 % for pre-plant soil fumigation (Johnson et al. 2012). Post-harvest products such as dry-cured ham and stored food were part of the CUEs given by the EPA (US EPA 2012). Methyl bromide is geographically available for use by ham producers in the southeast of the United States, as well as pre-plant for strawberry growers in California (CDPR 2011, EPA 2013). As a result, the EPA estimates that 5 % of the current stockpile will be usable in 2016. As of December 31, 2014, there were 158,121 kg of existing stocks and as a result, EPA will reduce new output by 7,906 kg in 2016 (GPO 2015).

With the phase out of methyl bromide, other fumigants such as sulfuryl fluoride and phosphine have been experimented on to ascertain their effectiveness and viability (Zhao et al. 2016). Experimental research to find alternatives to control *T. putrescentiae* started in the 2007's (García 2004, Graham et al. 2012). The fumigants that have been tested although having some potential of controlling ham mites also posed other issues that were not convenient to the ham industries.

ProFume®, brand name for sulfuryl fluoride, was created to replace methyl bromide in post-harvest applications (Phillips et al. 2012). Sulfuryl fluoride (SF) is a registered alternative fumigant which is safe to be used on stored products, and dry-cured ham has been included on the label since 2005 (EPA 2005). SF is known to effectively control all life stages of many stored product pests, although it does have issues controlling egg stages of some species (NPMA 1998). However, Phillips et al. (2008) demonstrated that although sulfuryl fluoride was effective against most of ham mite life stages, but eggs were not killed even at higher concentrations three times the label rate. Sekhon et al. (2008) also discovered that after fumigating with sulfuryl fluoride, there may be a low level of residue which may affect the legality and aroma/taste of dry cured

ham. Sensory testing will be required to determine whether a flavor is present or not after fumigation.

Another fumigant that was promising and approved for fumigating stored products, including dry-cured ham, is phosphine. The most significant benefits of phosphine are its ease of use, low cost, and potency. For stored products, the maximum permitted phosphine residue concentration is 0.01 ppm (USDA 1999). Phosphine does not degrade the ozone layer following diffusion into the environment, and decomposes into harmless chemicals (Chaudhry 1997, Zuryn et al. 2008). In experiments conducted by Sekhon et al. (2010) and Phillips et al. (2011), phosphine killed all life stages of ham mites at concentrations of 400 ppm and 1000 ppm after exposure for 48 hours. Sekhon et al. (2010) found phosphine had residual concentrations in dry cured ham after its fumigation, but the values were well below the approved levels for human consumption and had not impact on the ham pieces. Although phosphine caused 100 % mortality of ham mites, it is highly corrosive to metal. After fumigating with a phosphine concentration of 1600 ppm for 48 hours in a shipping container (36000 cubic feet) corrosion of electrical switches and appliances was reported (Zhao et al. 2012). This limits the application of this fumigant in ham aging houses.

Other gases such as ozone and carbon dioxide, and the use of controlled atmospheres, have been evaluated for control of ham mites. Ozone is permitted for use in the United States as a disinfectant in food processing industries (Guzel-Seydim et al. 2004). When ozone was used in the control of *T. putrescentiae*, all life stages were killed at of 175 ppm (Hansan et al. 2016).

Ozone did not leave any residues since it naturally degrades into oxygen. However, the disadvantage is that it is unable to enter porous surfaces under which ham mites are known to

hide. Again, due to its nature, it has a potential to cause oxidation of the food product which may negatively impact the flavor of the dry cured ham (Sekhon et al. 2010b).

Carbon dioxide (CO₂) can be used as a fumigant to control a variety of insect pests and does not leave behind any residue (Hasan et al. 2016). The effectiveness of CO₂ relies on maintaining an increased CO₂ concentration in the environment until all insects die. Hence, a leak-proof system is required. The duration of time spent exposed is determined by CO₂ concentration and ambient temperature (Page et al. 2002). All life stages of ham mites died after 144 hours of exposure to 60 percent CO₂ (Hasan et al. 2016). Increased CO₂ pressure would likely improve treatment efficacy, but it would also raise treatment costs, which is a disadvantage of this strategy (Riudavets et al. 2010). Disadvantages of CO₂ application include challenges connected to the required extended exposure period, which disrupts the production process, as well as a safety danger for persons working near ham aging rooms due to high CO₂ concentration. To achieve a high level of fumigant concentration and insect mortality, highly airtight constructions are required. Furthermore, CO₂ is costly, and vast amounts of a greenhouse gas must be released into the atmosphere, which has its own set of environmental consequences (Phillips et al. 2012, Hasan et al. 2016).

Other Integrated Pest Management Practices for Ham Mites

Integrated pest management is necessary to prevent ham mites from infesting dry-cured ham aging rooms since the use of multiple methods together can effectively control ham mites. The use of methyl bromide phasing out of use in ham industries, has caused researchers to conduct experiments to identify effective alternatives to methyl bromide in dry-cured ham plants. Other alternatives would have to be used once the supplies of the pre-phase-out methyl bromide in the US were depleted (US EPA 2017). It is therefore imperative to research alternative

substances and practices to methyl bromide which are safe, practicable, cost-effective, and successful to control ham mites.

Monitoring of *T. putrescentiae*

Monitoring can inform facility managers to changes in mite populations, which can be used as one component in pest management decisions and determining the impact of fumigation or other pest control measures (Amoah et al. 2016). Traps can also be used to estimate the mite population and determine when it's time to take action. Commercial mite traps are available in England (Wakefield and Dunn 2005). To evaluate mite populations in several dry cured ham companies in the Southeastern United States, Amoah et al. (2016) used a food baited mite trap similar to the "BT Trap" that was made in England (Thind 2005) and showed number of ham mites ranged from zero to several hundred per week over the course of two years. It is important to plan to take corrective actions if trap captures indicate high mite population and observation samples surpass a threshold limit (Amoah et al. 2017).

Sanitation

Another important factor in reducing ham mites' abundance in dry-cured ham plants is sanitation. Good sanitation practices and Sanitation Standard Operating Procedures (SSOPs) must be established and followed. Components of sanitation include sweep out and clean floor area with warm water, vacuum, scrubbed surfaces with soapy water, replace wooden racks, sanitize aging room, seal cracks and crevices to prevent pests from hiding and limiting employee access to the aging room (Schuler et al. 1999, Campbell et al. 2021). These are the main methods for keeping ham mites and other storage pests away from pieces of meat, trimmings, and oil that may be left on the floors and shelves of an aging room

Food-Safe Compounds

Food-safe chemicals that are registered to repel or kill arthropod pests have been tested on ham mites by scientists in their effort to alleviate the problem of mite infestations in an aging room. Several different food-safe compounds, such as propylene glycol (1, 2-propanediol), lard, ethoxyquin, and, butylated hydroxytoluene, were tested for their efficacy to control a population of ham mites after 14 days. It was discovered that several of these compounds had potential to manage ham mites since their populations were reduced drastically after feeding on treated ham (Abbar et al. 2016a, Campbell et al. 2018, and Campbell et al. 2020). In a behavioral trial, Zhang et al. (2017) discovered that 95% of ham mites preferred ham cubes wrapped in untreated nets to those wrapped in nets treated with food-safe substance combinations such as xanthum gum, carrageenan, propylene glycol alginate, and propylene glycol. Further work with nets treated with these food-safe substance to cover full hams are being evaluated to determine their efficacy and practicality in a dry-cured-ham plant or commercial aging rooms.

Use of Different Temperature Ranges and Light Types

Ham mites are unable to resist high temperatures. Hence heat can be used to kill ham mites after removing all the hams from the aging room by keeping each room at 40 °C for 4 days, 42 °C for 2 days, or 56 °C for 1 day (Abbar et al. 2016b). Alternatively, ham mites are unable to withstand cold temperatures in an environment. By cooling the room to at -15 °C for 12 hours, -10 °C for 1 day, -7 °C for 40 hours, or -2 °C for 2 days, ham mites can be killed by the cold (Abbar et al., 2016b). Contaminated hams can be placed in a walk-in freezer for 24 hours at -15 °C to kill ham mites.

Ham mites like darkness and avoid bright lights, such as red or white incandescent, fluorescent, or LED bulbs. Hence the use of lights 24 hours a day, seven days a week could

possibly keep ham mites away from hams in an aging room (Campbell et al. 2021). More research to investigate lights in a simulated aging room is ongoing.

Residual Effect of Registered Pesticides

Pesticides are used to control mite pest species. However, because of ham mites' short life cycle, high selection pressure, and dispersal potential, acaricide resistance and population outbreaks are common concerns (Freitag and Kells 2013). Studies have shown that pesticides that are highly toxic to house dust mites *Dermatophagoides pteronyssinus* and *D. farina* are also effective at controlling *T. putrescentiae* (Stara' et al. 2011a). Stara' et al. (2011b) determined that a multi-compound acaricide based on a combination of benzyl-benzoate, permethrin, and pyriproxyfen was effective at controlling ham mites. It was discovered that the toxic activity of chlophenapyr could last on metal, concrete and wood surfaces even after the eighth week from time of application and still kill 100 % of ham mites on these surfaces (Abbar et al. 2016c).

Enhancing and mixing of currently available products and tactics to control ham mites in dry-cured ham operations, as well as putting forward environmentally and customer-friendly IPM programs as an alternative to methyl bromide, are still being evaluated. The main objective this study was to look into different mite control approaches for *T. putrescentiae* in order to reduce mite populations and limit the incidence of mites on dry-cured hams and surfaces in the processing and aging room.

Objectives

- i. Assess the effectiveness of various food-safe compounds in repelling ham mites by investigating how the population of ham mites would be affected by conducting choice and forced feeding behavioral assays and also to ascertain ham mites' orientation when ham or surface is treated with these essential oils.

- ii. Assess the efficacy of some selected registered acaricides and to evaluate their toxicity against ham mites by screening and to investigate the residual effect of acaricides after applying on three different surfaces: metal, wood and concrete.
- iii. Determine how liquid and sticky barriers will affect the movement of mites on a small-scale experimental arena in the laboratory and to determine the effect of different selected physical barriers on ham movements to dry-cured ham in a simulated aging room
- iv. Develop extension article that will summarize all proposed integrated pest management tools experimented on to control *T. putrescentiae* to educate ham producers and pet food industries as well as food storage and processing facilities.

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Chapter 2 - Natural and Synthetic Repellents for Pest Management of the storage mite *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes:Acaridae)

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Introduction

Tyrophagus putrescentiae (Schrank) (Acarina: Sarcoptiformes), known as the ham mite, cheese mite, or mold mite, is a cosmopolitan arthropod pest that infests a large number of durable stored foods and processed commodities with high-fat and high-protein content and moisture contents from 15 to 45% (Erban et al. 2015, Al-Nasser 2017). Ham mites usually live on the surfaces of high-value stored products such as dry-cured ham, other dried meats, cheeses, nuts, dried fruits, spices, cultured cheeses, and semi-moist pet foods, and they sometimes penetrate into the food to cause economic losses (Atthanassiou et al. 2002, Ayguna et al. 2007, Rentfrow et al. 2008, Athanassiou et al. 2011).

Southern dry cured ham is aged to achieve the desired taste, flavor, and texture, but mite infestation during aging can lead to regulatory action from meat inspectors (Marriot and Schilling 2004). Over the years, mites have been problematic but effectively controlled solely by using methyl bromide, which served as a fast-acting and very effective fumigant (Sekhon et al. 2010). However, methyl bromide is being phased out of use and is not available

in most industrialized countries at this time because of its detrimental action to the ozone layer of the earth's atmosphere (EPA 2006).

The use of synthetic chemical pesticides to control insects and other arthropod pests is decreasing, since concerns have been raised based on human health, environmental effects, and pesticide resistance. Therefore, alternatives that are natural products and safe for both humans and the environment are being evaluated for pest control applications (Nerio et al. 2010). Consequently, a search into these natural products as alternatives to control insects and other arthropods can be useful. Safe natural products include essential oils, which are naturally occurring defensive compounds in plants that include monoterpenes, sesquiterpenes, and diterpenes from plants that serve as protective allomones to repel and intoxicate phytophagous insect. Some essential oils are known for their acaricidal effects (Sanchez-Ramoz and Castanera 2000), which led us to investigate these compounds as repellents for ham mites.

Methods such as food-safe compounds incorporated in ham nets to prevent infestation of hams and traps to detect and monitor mite populations over space can serve as tools for integrated pest management for ham mites (Abbar et al. 2016, Amoah et al. 2016, Zhao et al. 2016, Zhang et al. 2017). Abbar et al. (2016) tested different food-safe compounds such as propylene glycol (1, 2-propanediol), lard, ethoxyquin, and butylated hydroxytoluene in controlling experimental populations of ham mites after 14 days (Abbar et al. 2016). Recent work by Zhang et al. 2017 demonstrated that 95% of ham mites oriented to ham cubes wrapped in untreated nets as compared to cubes wrapped with nets treated with food-safe repellents such as xanthan gum, carrageenan, propylene glycol alginate, and propylene glycol in a behavioral experiment at different concentrations.

This present study focuses on using terpenoids that occur naturally in many vascular plants as well as food-safe synthetic repellents to deter and mitigate ham mite populations for eventual use in commercial ham aging rooms. A fatty acid blend, C8910, is made up of three medium-chain fatty acids with C8, C9, and C10 normal chain lengths mixed in equal parts, which occur in some plants and were elucidated earlier as a safe pesticide (Reifenrath 2010). Octanoic and decanoic acids are natural derivatives of palm kernel oil or coconut oil by the process of steam hydrolysis, while nonanoic acid is derived from tallow or from the action of lightening and ambient oxygen and oleic acid in the film of water droplets, which are less expensive and easy to access (King et al. 2009, Dunford et al. 2014). C8910 is used as both a repellent and an insecticide against susceptible and resistant strains of insects and other arthropods (Samuel et al. 2015). The commercial formulation of C8910 is repellent against biting and non-biting flies such as mosquitoes and acarids such as ticks (Mullen et al. 2009). Stractor, Inc. (Richmond, CA, USA) produces C8910, which is U.S. FDA approved and categorized as “generally recognized as safe” (Dunford et al. 2014). According to Mao and Henderson 2010, nootkatone is a natural sesquiterpene that is obtained from grapefruit and has contact toxicity and repellency to some stored product insect pests such as the maize and rice weevils, *Sitophilus zeamais* and *S. oryzae* (Coleoptera: Curculionidae). Ticks, *Ixodes ricinus* (Linnaeus) (Ixodida: Ixodidae), have been effectively repelled by oxygenated compounds such as citronella, cinnamyl alcohol, geraniol, and α -pinene, which were isolated from the essential oil from flowers of the *Dianthus caryophyllum* and *Artemisia abrotanum* plant (Tunon et al. 2006). Carvacrol, geraniol, and citronella were found to repel *Anopheles gambiae* (Diptera) successfully at lower concentrations (Omolo et al. 2004, Odalo et al. 2005). It was also discovered that the commercial synthetic insect repellent DEET (N, N-

Diethyl-3-toluamide) is not only a broad-spectrum repellent but is very effective in repelling mosquitoes, since it is persistent in the skin of humans, unlike other synthetic compounds (Isman 2006). Citronella is a bio-pesticide that is safe for human use and has been approved by the United States Environmental Protection Agency since 1948 (EPA 1997). Citronella is used for protection against mosquito bites and prevents the invasion of stored food products by arthropod pests. Interestingly, citronella is more effective as a repellent than DEET in many applications (Osmani et al. 1972, Suwonkerd and Tantrarongron 1994, Barnerd and Xue 2004, Amer and Mehlhorn 2006). Icaridin is another compound that has been evaluated for its ability to repel arthropod pests. One study suggested that Icaridin could be a very good substitute to DEET, since it repelled two species of ticks in a laboratory experiment (Büchel et al. 2015). We chose to include DEET and Icaridin in our studies as mite repellents because they are safe for humans, they are not natural products and thus are included as comparisons to natural products, and they have not been evaluated as repellents for stored product pests. These synthetics are considered food-safe and likely could be approved by food regulatory agencies to repel ham mites from hams and other foods in the future.

In order to ameliorate the problem of ham mites from dry-cured ham, the use of food-safe compounds which are registered to either repel or kill these pests is drawing research attention (Abbar et al. 2016, Zhang et al. 2017). Therefore, the objective of this study was to assess the effectiveness of some food-safe compounds and essential oils as allomonones for repelling ham mites. Additional experiments on the oviposition, infestation, and population growth of mites exposed to food-safe repellent-treated ham pieces were conducted using the most repellent compounds.

Materials and Methods

Mite Cultures and Ham Used

Tyrophagus putrescentiae (ham mites) were reared in glass jars (85 mm diameter, 160 mm height, volume of 917 mL) containing mite diet that were firmly sealed with labelled filter paper (Fisher Filter paper, diameter 7.0 cm, Pittsburgh, PA 15275, USA) in a metal lid ring. The diet was prepared as described in previous research projects in our laboratory (Abbar et al. 2016, Amoah et al. 2016). The ingredients in the diet for rearing the mites included 5 g each of agar (ICN Biomedicals, Inc. Aurora, OH, USA); yeast (MP Biomedicals, LLC, Santa Ana, CA, USA); alphacel (ICN Biomedicals, Inc.); and mixed vitamins (Vanderzant modification vitamin mixture for insect diet; MP Biomedicals, LLC, Santa Ana, CA, USA). Other ingredients included 160 g of commercial dry dog food that was ground, and the liquids glycerol (Fisher BioReagents, Fisher Scientific, Pittsburgh, PA, USA), antifungal salt solution in ethanol (methyl-p-hydroxybenzoate, 15:85 g mL⁻¹) (ICN Biomedicals, Inc.), and water in a ratio of 25:25:475 mL. These ingredients were mixed and brought to boil for 30 min. Then, approximately 30 mL of the cooked mixture was added to each jar that contained approximately 50 g of unground dog food kibble in the rearing jars. The jars were cooled to room temperature, and then mites were introduced to the new cultures. The cultures were firmly sealed and placed in plastic containers that were filled halfway up with soapy water to prevent the escape of mites. The plastic containers with the jars were placed in an incubator at 25 °C and 70% RH in total darkness. Hams used in this study were acquired as whole country hams (6–8 kg) that had been aged for a minimum of 3 months from a commercial dry cured ham company in Tennessee.

Chemicals

Table 2.1. Lists the compounds studied and their suppliers.

Repellency of Test Compounds Using Two-Choice Orientation Assay

Two-choice behavioral tests were conducted by using glass Petri dishes (90 × 20 mm glass Petri dishes) as a behavioral assay arena to test the orientation of mites. Test compounds in acetone were on one side of the dish, and the other side contained only acetone as the untreated control. The floors of the glass Petri dishes were divided into two equal halves by drawing a line through the middle with a grease pencil. This also prevented the test compound solution from migrating to the control arena of the Petri dish before the solvent evaporated. Compounds used included the fatty acid blend of C8910, Icaridin, DEET, citronella oil, carvacrol, nootkatone, and geraniol. Application rates of 0.001, 0.02, 0.04, 0.06, 0.08, and 0.1 mg/cm² were used for each test compound. Approximately 100 ham mites of mixed life stages were placed at the center of the Petri dish to determine which side the mites oriented toward. Mites were added by first placing a small rectangular piece of white paper with an area of 6 mm² inside an active mite colony for 5 sec while being held with forceps. Mobile mites crawled onto the paper, and then, the paper was lifted out of the rearing jar and placed in the center of a given Petri dish floor. Mites were prevented from leaving the assay Petri dishes by applying a thin layer of vacuum grease along the inside upper 5 mm of the vertical sidewall of the dish. Once dishes were set up, repellency assays were conducted in a room with complete darkness at a temperature of 25 °C and 70% RH. Mites were allowed to move in the Petri dish for 30 min and then were counted based on the half of the arena they oriented to, which were either the treatment or the control.

Mite Orientation and Oviposition Bioassay

Initial experiments on mite repellency in Petri dish arenas (methods above) indicated that C8910, nootkatone, and geraniol were very effective at repelling mites and were examined

further for how they may affect mite orientation to and oviposition on ham meat. C8910, nootkatone, and geraniol were evaluated in a similar behavioral assay using 90×20 mm glass Petri dishes (Pyrex[®], Frankfurt, Germany) as arenas, each with a 90 mm diameter circular piece of black construction paper under the floor of each dish to allow for observation of the small white mites. Ham cubes of $5 \times 5 \times 5$ mm (125 mm^3) were used in two-choice assays. The ham cubes were cut from the same section of the whole dry-cured ham, with a new ham being used for each experiment. Three circles of 18 mm diameter were drawn with pencil on the black construction paper and centered along a line passing through the center of the paper, with one circle in the middle of the floor and one circle each at a distance of 5 mm from the side wall. Then, the circular construction paper was placed beneath the Petri dish and held in place with double-sided tape. Concentrations of 25, 50, 75, 100, and 150 ppm ($\mu\text{g/mL}$) of each test compound were made using acetone as the solvent. Treatment ham cubes were dipped in the various solutions, and the control cubes were dipped in acetone only, for 1 min, and then allowed to air dry for 60 min. For each of the five bioassay arenas for each treatment, one ham cube was treated with the test compound, and the control solvent-treated ham cubes were placed in each of the circles closest to the dish wall. Thirty adult mites of mixed sexes were released into the middle circle of each assay arena. Mites were prevented from leaving the bioassay dishes by applying a thin layer of vacuum grease along the inside upper 5 mm of the vertical wall of the Petri dish. The Petri dishes were covered and left on a bench in total darkness at 25°C and 70% RH for 48 h without any disturbances until data collection. Mites that oriented to each of the ham cubes, which included those on the ham surface and on the floor within the circle outlining the ham cube were counted under red light in the

dark room at 1, 2, 3, 24, and 48 h. Then, mite eggs laid by the end of this 48 h period were counted under a binocular dissecting microscope (200 × X) and recorded.

Mite Population Growth

The population growth of mites on ham that was treated with test compounds at different concentrations was determined. The food-safe compound, C8910, and the essential oil terpenoids, citronella, carvacrol, geraniol, and nootkatone were evaluated in separate experiments using a completely randomized design of five replicates per concentration (treatment) of a test compound as done previously (Abbar et al., 2016).

Dry-cured ham cubes (25 × 25 × 25 mm) were dipped in a given concentration of the test compound; water or acetone alone (depending on the compound) were used as controls. Concentrations of 1 % and 10 % of test compound were compared to the solvent control. Five ham cubes were placed in 200 mL of the test solution in a 500 mL beaker for 1 min, removed and left on filter paper in an open 90 cm glass Petri dish to dry for 60 min. Then, treated ham cubes were placed separately inside small glass Mason jars (216 mL, 65 mm diameter, 55 mm height; Ball Corp., Broomfield, CO, USA), and 20 adult mites of mixed sexes were introduced for infestation; a similar procedure was followed for the controls. Ham mites were prevented from leaving the assay jars by applying a thin layer of vacuum grease along the inside upper side wall 15 mm from the bottom. The jars were covered with their screw-on ring lid with a circular piece of filter paper for ventilation (in place of the metal lid intended for such jars); then, they were placed in opened plastic container filled half-way with soapy water to prevent any escaping mites from contaminating the laboratory. Incubation was done for 14 days at 25 °C and 70 % RH with 16:8 h L: D

photoperiods. Live mites and progeny (mobile mites of adults and immatures) in each jar were counted using a microscope (as above).

Statistical Analyses

Repellency index (RI) for the initial two-choice orientation bioassays was calculated using:

$$RI = ((N_c - N_t) / (N_c + N_t)) \times 100$$
, whereby N_c = the number of mites on the control side and N_t = the number of mites on the treated side (Koko and Chandrapatyra 2009).

Data were statistically analyzed using SAS software 9.4 (version 2012). We subjected the RI proportional data from behavioral assays using the arcsine transformation prior to statistical analyses. We used one-way ANOVA to determine the significant effects of the treatment concentrations on mite orientation. Differences between the treatment and control were determined with the Ryan–Einot–Gabriel–Welsch and Quot (REGWQ) test for multiple comparisons at a probability of $p < 0.05$. The residency of mites on ham cubes with different coatings and within the circles were subjected to a two-way ANOVA for each observation time at different concentrations. Mean RIs at different observation times over 48 h and for concentrations of given compounds were separated by the honestly significant difference Tukey–Kramer test when the F-test of the ANOVA was significant at $p < 0.05$. With the oviposition study, statistical analyses for differences between treatment and controls for the percentage of eggs laid on each were determined with the student's t -test following arcsine transformation. For the population growth experiment, data were analyzed with SAS for significant effects of test compound concentration on mite numbers. Then, the REGWQ test was used to separate population growth means for multiple

comparisons at a probability of $p < 0.05$. We used SigmaPlot Version 11 to draw graphs for results as needed after the data were analyzed.

Results

Two-Choice Orientation Assays

The orientation assays demonstrated that mites oriented more toward untreated side of the Petri dish than to the treated side as indicated by positive RIs in all cases. However, depending on the compound tested, some compounds appeared more repellent than others (Figures 2.1 and 2.2). It was also apparent that the concentration of a compound used had an effect on mite orientation, since as the chemical concentration increased, more mites moved away from the treated side, representing a concentration-dependent response. With the terpenoids on the glass surfaces, geraniol had a high RI of 88.9 % at a concentration of 0.04 mg/cm², which was not different ($p > 0.05$) from geraniol at 0.1 mg/cm² with an RI of 96.3 %. The repellency of the other terpenoids at concentrations of 0.08 and 0.1 mg/cm² for carvacrol, citronella, and nootkatone with RIs of 77.5 % and 92.5 %; 74.5 % and 84.0 %; and 73.2 % and 82.3 %, respectively (Figure 2.1). The synthetic compounds C8910, DEET, and icaridin were less repellent than the natural products, with the highest repellency indices at 0.1 mg/cm². The three synthetic compounds C8910, DEET and icaridin had mean RIs of 85.6, 73.1, and 68.2 %, respectively at 0.1 mg/cm², which were lower than the RIs of the essential oils (Fig 2.2).

Mite Orientation and Oviposition on Treated Ham Cubes

Similar to results from the dish arena assays, the orientation of mites to ham pieces was less as the concentration of a given repellent increased ($p < 0.05$), regardless of exposure time (Table 2.2). All three compounds were repellent to some degree at all concentrations,

and many of them showed no significant differences of RI over the five different time periods of the assays.

With regard to the number of eggs laid within the period of 48 h, the raw data showed a range of 1–50 eggs for the solvent-treated controls and 0–27 eggs for the treated ham cubes. Nootkatone-treated cubes of ham had the least number of eggs with a range of 0–4, followed by geraniol with 0–7 and C8910 had 1–27 eggs, respectively. The proportion of eggs laid on untreated ham cubes was always significantly higher than the proportion laid on untreated cubes (Table 3). There were no eggs, to very few, laid on ham pieces treated with either nootkatone or geraniol for any of the five concentrations tested. Therefore, these two terpenes could serve as good repellents to prevent the infestation of dried ham.

Mite Reproduction Assay

This study focused on the population growth of ham mites on ham cubes when they were treated with either C8910, nootkatone, geraniol, citronella, or carvacrol, all of which were effective at repelling mites (Figures 2.1 and 2.2; Tables 2.2 and 2.3). Mite numbers produced on ham cubes dipped in solutions of C8910 and the four terpenoids were significantly lower in all treatments than the number of mites produced on the solvent controls (Figure 2.3). The terpenoids at different concentrations caused a reduction in mite reproduction after 14 days. Mite numbers on ham cubes treated with either a 10 % concentration of nootkatone, geraniol, C8910, citronella, or carvacrol in acetone were significantly reduced to means (and SEs) of 51.6 (3.67), 78.8 (2.99), 12.4 (4.06), 8.2 (1.43), and 4.4 (0.74) respectively, when compared to controls. There were no significant differences observed between 1 % and 10 % concentrations in citronella and carvacrol, whereas the other oils differed significantly at these concentrations. This makes citronella and carvacrol the most effective at reducing the

population of mites when used at dosages of 1 or 10 %. Generally, all essential oils and the fatty acid mixtures reduced mite population growth substantially when applied on ham pieces.

Discussion

This research provides evidence that some naturally occurring plant compounds and other common synthetic repellents will repel and reduce the population growth of ham mites when applied at various concentrations in the laboratory. In recent times, a common way to study the behavior and ecology of animals is by choice bioassays (Raffia et al. 2002). Choice bioassays were employed for the study of ham mite behavior in this research. The results of the relationship between ham mites and the different food-safe compounds demonstrated that ham mites readily moved away in the presence of these test compounds. In the behavioral bioassays, the different essential oils had strong repellency against *T. putrescentiae* on glass surfaces. Ham mites oriented to the control side of the Petri dishes anytime they were allowed to stay undisturbed on the bench for 30 min. The few that managed to cross the line that separated the control and treated sides retracted themselves quickly to avoid the treated side when viewed under the microscope. The ability of essential oils used in these studies to repel other stored product insect pests from commodities has been widely studied e.g., (Regnault-Roger 1997, Suelen et al. 2016, Ramadan et al. 2020). Not only are essential oils used to repel insects from food commodities, they are also used for health purposes to prevent malaria (Kalita et al. 2014), since they repel mosquitoes from biting humans (Muller et al. 2009).

The essential oil geraniol was the most effective compound for repelling mites of those tested, with an RI of 96.3 %, which indicates that geraniol has potential for application in the management of ham mites. Past studies confirm that geraniol strongly repels both red flour beetles and booklice in comparison to citronellol and limonene when applied to control these

stored product insect pests (Zhang et al. 2017, Zhang et al. 2011). Another study found that mechanical diffusers with geraniol had a 97 % repellent rate compared to other essential oils such as citronella and linalool diffused for repelling mosquitoes (Muller et al. 2009). There is substantial information of geraniol having a higher tendency to repel arthropods than other essential oils (Chen and Viljoen 2010). The results from our work also indicate that geraniol is more repellent against ham mites than other essential oils tested, which is similar to what has been reported for mosquitoes by Hao et al. 2008.

Citronella and carvacrol have the potential to effectively repel mites at higher concentrations. Citronella has been used in food packaging to protect commodities from insect pests. Citronella protected treated food-containing cartons for 2-week periods and minimized the infestation of insects by 50 % at concentrations as low as 0.2 g/m^2 of carton board (Wong 2005). Likewise, citronella incorporated in food packages was found to effectively repel the red flour beetle, *Tribolium castaneum* (Licciardello et al. 2013). The results described here indicate an RI of 84.0 % when ham mites were exposed to a glass surface treated with citronella oil at a concentration of 0.1 mg/cm^2 .

Carvacrol is mostly known for its toxicity against arthropod pests more so than its repellency potential; however, when used in small concentrations, it can protect commodities by repelling arthropod pests (Carroll et al. 2017). The results from our study suggested a significantly higher repellency index of 92.5 % at a concentration of 0.1 mg/cm^2 , which indicates potential for use as a repellent to manage mites. In one study Carroll et al. (2017), carvacrol was the best repellent of all tested compounds against ticks and mosquitoes. Although carvacrol is known to be effective, it easily loses its potential of repelling insect pests after 7 days (Jordan et al. 2012).

Nootkatone was less effective at repelling mites when compared to the other terpenes that were tested. However, due to nootkatone's mild odor in comparison to other essential oils, it is recommended for consideration in further experiments, since it protected ham pieces from ham mites by reducing the number of eggs laid by the mites. Earlier work with nootkatone reported repellency percentages that ranged from 46.3 to 93.1 % and 39.2 to 67.3 % against maize and rice weevils, respectively, as nootkatone concentrations increased from 0.05 to 5 % (w/v) in methanol (Mao and Henderson 2010). It was concluded that nootkatone was a good repellent candidate for insect pest management. Similarly, when nootkatone was used in the present study, the highest repellency index was achieved at the highest concentration of 0.1 mg/cm with an RI of 82.3 %.

The U.S. Environmental Protection Agency (EPA)-approved repellents that are used to repel disease-causing arthropods such as flies, ticks, and mosquitoes were evaluated here for their efficacy at controlling mites. Icaridin and DEET are well-known repellents for application to human skin that have the potential to repel biting insects and arachnid pests such as mosquitoes and ticks. According to our data, C8910, which is EPA-approved as a fly repellent on livestock, significantly repelled ham mites at higher concentrations, while DEET performed poorly as a mite repellent. C8910 is commercially marketed for use on humans and livestock in southern regions of Africa to control biting flies (Reifenrath 2010). The present study indicated that the repellents DEET and icaridin were the least effective at repelling ham mites compared to all the other compounds tested (Figure 2.2). The exact mode of action for biting insects and ticks to not contact or move away from synthetic repellents is not understood, although there is evidence that repellents work through interactions with odorant receptors and gustatory receptors in arthropods (Dickens and Bohbot 2013)

Our orientation and oviposition experiment found that mites avoid ham cubes treated with C8910, nootkatone, and geraniol, and they were then drawn to oviposit on the untreated ham pieces (Table 2.3). These three compounds lacked efficacy at repelling mites at the lower concentrations of 25 and 50 ppm. Recent work suggests that the quality of the plant material from which an essential oil is obtained and its composition of volatile natural products may be different than that of the purified essential oil compounds (Naeem et al. 2018). This was evident in our results from the geraniol and nootkatone-treated ham cubes. Pieces of ham were available to the mites on either side of our test arenas (treated or untreated) in this study, but it was clear that ham mites tended to stay on the untreated ham, and we observed that the adult mites were not dying when near high concentrations of these compounds for up to 48 h. It was clear that the treated ham affected the number of eggs laid on untreated ham cubes for up to two days after treatment (Table 2.3). This choice for oviposition on untreated ham cubes was the same as in our previous work (Abbar et al. 2016).

Mites were apparently repelled by the smell of the treatment compounds and thereby settled on the untreated ham for oviposition on the opposite side of the Petri dish. Repellency and negative orientation is probably adaptive because nymphs from any eggs laid on or near ham pieces treated with repellents may fail to eclose or the nymphs might die soon after eclosion. The activity of these volatile mixtures of natural products is dependent mainly on the type and quality of plant material that the oil is derived from and the process by which the manufacturer extracted the product (Sarkic and Stappen 2018). With respect to C8910, the blend of synthetic short-chain fatty acids, there was relatively good reduction in the fecundity when pieces of ham were treated with it. Aside from these short chain fatty acids, other fatty acids have been studied previously. Caproic (six carbons) and propionic

(three carbons) acids at 1 and 2 % (by weight) caused a 100 % reduction in the oviposition of *T. putrescentiae* from ham pieces (Rodriguez 1972). However, these compounds applied to treated ham nets gave netted ham slices a moderate difference from untreated controls for a human taste panel. However, the C8910 had potential for achieving better consumer acceptability when mixed with other food-safe oils (Rogers et al. 2020).

There has been very little work done to investigate the repellency of these food-safe plant-derived compounds against *T. putrescentiae*, although a lot of work has been conducted on the efficacy of these compounds against several species of stored product insect pests (Neiro et al. 2010, Kalita et al. 2014). The most effective essential oils at repelling mites were further investigated to assess their acaricidal effect on mite populations on ham. Results with terpenes as mite repellents are in agreement with earlier work on insects. (Pinheiro et al. 2013, Park et al. 2017) that reported results of higher toxicity of citronella and carvacrol against insect pests. Similar results were found when these compounds were used in controlling insect pests of stored grains (Kalita et al. 2014).

In the population growth study, the results indicated that C8910, citronella, and carvacrol prevented the growth of ham mite populations over 14 days of storage. The low number of ham mites on treated ham cubes at 14 days suggests that mites may have been inhibited from laying eggs on the treated ham, hence preventing population growth from the initial number of 20 mites inoculated on the ham pieces at a concentration of 10 % in the dip. Similar results were obtained in Abbar et al. (2016) when ham cubes were dipped in other safe and approved food additives, which suggests that the application of various food-safe compounds may be an effective way to prevent infestation by ham mites on dry-cured hams. Abbar et al. (2016) tested compounds not known to be directly toxic to mites and insects,

so the hypothesis of reduced oviposition leading to reduced population growth may be valid. However, in our work using the same population growth assay, it is possible that the low population on treated ham cubes resulted from the mortality of some or all of the initial 20 mites in a jar caused by the toxic volatiles of these repellents.

This possibility requires further research. A recent study from our group showed that the addition of C8910 to nets used for covering and hanging country hams during aging clearly had reduced mite numbers on ham compared to ham in nets with no C8910 (Rogers et al. 2020). Although C8910 had better efficacy at inhibiting *T. putrescentiae* population growth in the present work, its strong smell after application needs further study for consumer suitability. Likewise, hams age in nets containing the essential oils found effective at repelling mites in the current study would need to be evaluated for acceptability by human taste panels. Instead of the use of safe repellents such as those studied here in nets used to hang and age hams, it may be effective to coat non-food structural surfaces (e.g., floors, walls, hanging racks, etc.) within an aging room to prevent mites from crawling onto aging hams for a distance of several meters. Future research should be done at both laboratory scale and commercial scale to determine if these natural and synthetic repellents can keep mites away from hams on aging racks. The use of safe repellents could be a viable preventive component of IPM for high-value dry-cured hams.

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Table 2.1. Synthetic food-safe compounds and essential oils that were evaluated for their efficacy at repelling and suppressing mite population growth on ham pieces

Category	Commercial Compound Name	Chemical Name or Natural Source	Supplier ²
Plant Terpenes	Carvacrol	Thyme, <i>Thymus</i> spp.	Sigma Aldrich
	Citronella oil ¹	Lemon grass, <i>Cymbopogon</i> spp.	Sigma Aldrich
	Geraniol	Rose oil, <i>Rosa</i> spp.	Sigma Aldrich
	Nootkatone	Grapefruit, <i>Citrus sinensis</i> x <i>C. maxima</i>	Sigma Aldrich
Synthetic repellents	C8910	Octanoic, Nonanoic Decanoic acid	Emery Oleochemical LLC
	DEET	N, N-Diethyl-meta-toluamide	Sigma Aldrich
	Icaridin	Icaridin	Sigma Aldrich

Terpenes that makes up citronella oil are as follows citronella (32-45%), geraniol (3-8%), citronella (11-155) and limonene (1-4%). ² Sigma Aldrich located in St. Louis Missouri, USA and Emery Oleochemical LLC located in Ohio, USA.

Table 2.2. Orientation of *T. putrescentiae* to dry-cured ham pieces coated with three different repellents vs. a water-control in laboratory two-choice bioassays. Mean repellency index, RI, ($\pm$ SE; n = 5) of mites orienting over time to ham pieces treated with repellent solutions of different concentrations (ppm).

Compound	Time (h)	Concentration (ppm)				
		25	50	75	100	150
C8910	1	20.0 (7.3) Bc	52.0 (9.8) Aab	38.7 (2.5) bc	68.0 (2.5) a	69.3 (4.5) a
	2	38.7 (4.9) ABb	66.7 (6.7) Aa	62.7 (3.4) a	73.3 (6.3) a	74.7 (5.3) a
	3	60.0 (3.7) Ab	74.7 (4.4) Aab	65.3 (6.5) ab	82.7 (2.7) a	74.7 (4.9) a
	24	26.7 (8.7) Bc	70.7 (12.8) Aab	41.3 (9.3) bc	60.0 (8.7) abc	89.3 (4.9) a
	48	28.6 (12.4) Bbc	13.2 (3.6) Bc	73.3 (11.4) a	53.7 (12.1) ab	87.0 (6.3) a
Nookatone	1	36.0 (5.4) Ba	50.7 (6.2) a	61.3 (7.7) a	68.0 (11.4) a	68.0 (9.0) a
	2	44.0 (6.5) ABc	58.7 (8.3) bc	62.7 (7.8) bc	86.7 (6.3) a	77.3 (3.4) ab
	3	42.7 (5.8) ABb	52.0 (6.5) ab	65.3 (6.8) ab	76.0 (7.5) a	78.7 (7.7) a
	24	45.3 (9.0) ABb	47.3 (12.8) b	48.0 (10.6) b	50.7 (17.8) b	82.8 (5.4) a
	48	56.3 (8.6) Ab	55.9 (13.5) b	57.4 (10.5) b	85.3 (3.9) ab	90.2 (4.8) a
Geraniol	1	62.7 (8.6) a	66.4 (8.9) a	71.8 (7.5) Aa	75.5 (2.6) a	84.7 (7.4) a
	2	45.2 (9.7) b	63.8 (7.6) ab	60.2 (7.8) ABab	70.5 (8.8) ab	82.3 (8.9) a
	3	57.4 (5.7) b	71.8 (9.1) ab	62.7 (8.0) ABab	70.4 (6.1) ab	89.5 (4.5) a
	24	49.2 (7.7) b	40.1 (6.1) b	33.1 (6.9) Bb	59.1 (11.6) ab	73.0 (4.4) a
	48	58.5 (5.8) a	66.6 (5.3) a	64.1 (8.7) Aa	81.5 (5.7) a	83.3 (7.6) a

Means followed by the same letter are not significantly different ($p > 0.05$). Lower case letters indicate significant differences among mean RIs at different concentrations in a row, while upper case letters indicate significant differences among RIs for each exposure time in a column for a given concentration. Exposure times with no upper-case letters did not have significant differences among RIs.

Table 2.3. Mean proportions of eggs laid by *T. putrescentiae* ($\pm$ SE; n = 5) on small dry-cured ham pieces treated with various food-safe compounds compared to untreated ham pieces after 48 h in a laboratory two-choice behavior bioassay. Student's t-tests following arcsine transformations of proportions were performed.

Compound	Concentration (ppm)	Mean ($\pm$ SE) Number of Eggs @ 48 h			
		Control	Treated	t-Value (df)	p-Value
C8910					
	25	64.7 (2.9)	35.3 (3.0)	7.0 (4)	<0.01
	50	62.2 (4.6)	37.8 (4.6)	3.6 (4)	<0.01
	75	90.2 (3.6)	9.8 (3.5)	15.8 (4)	<0.01
	100	78.9 (6.7)	21.1 (6.7)	6.1 (4)	<0.01
Nootkatone	150	90.9 (4.8)	9.1 (4.8)	12.1 (4)	<0.01
	25	76.7 (19.4)	3.3 (3.3)	3.7(4)	<0.02
	50	92.0 (8.0)	8.0 (8.0)	7.4 (4)	<0.01
	75	81.7 (8.9)	18.3 (8.9)	5.0 (4)	<0.01
	100	100.0 (0.0)	0.0 (0.0)	-	-
Geraniol	150	98.2(1.82)	1.8 (1.8)	37.5 (4)	<0.01
	25	92.5 (7.5)	7.5 (7.5)	8.0 (4)	<0.01
	50	96.7 (3.3)	3.3 (3.3)	19.8 (4)	<0.01
	75	91.0 (5.6)	9.0 (5.6)	10.4 (4)	<0.01
	100	96.7 (3.3)	3.3(3.3)	19.8 (4)	<0.01
	150	100.0 (0.0)	0.0 (0.0)	-	-

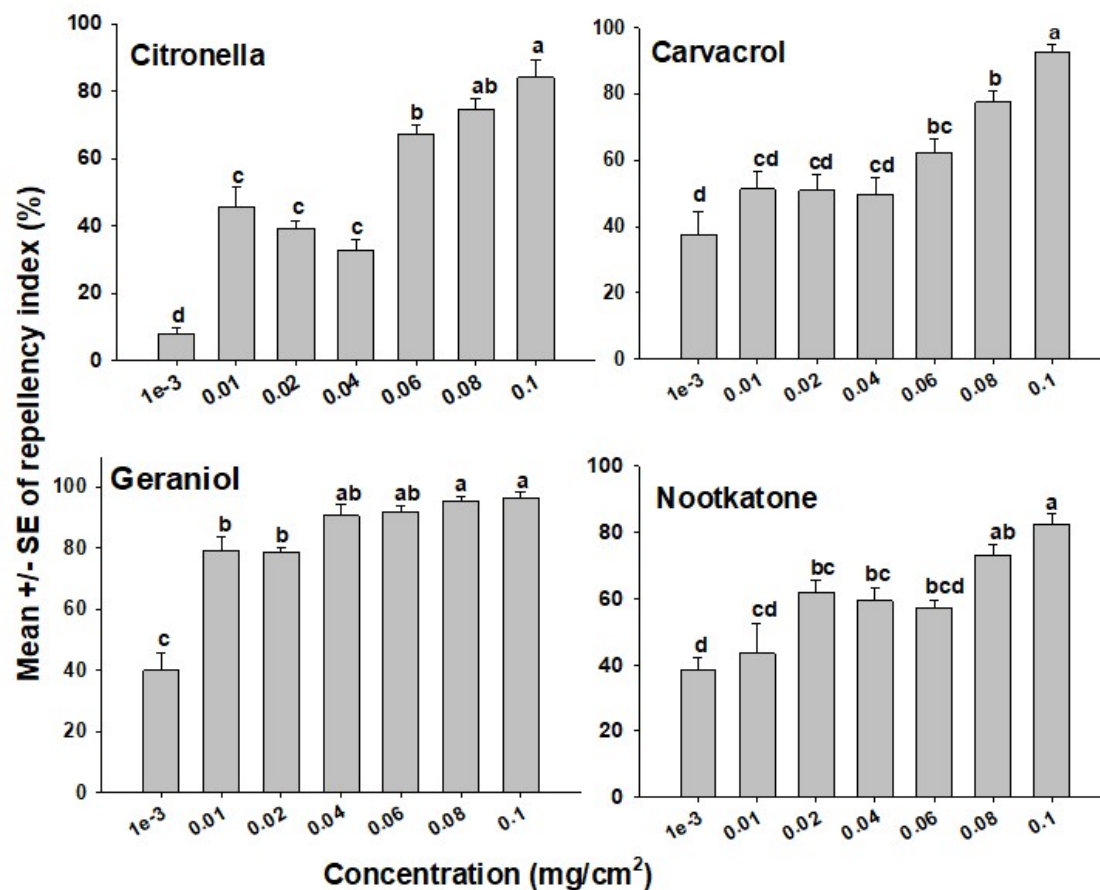


Figure 2.1. Orientation response of *T. putrescentiae* to glass surfaces treated with various terpenoids in a laboratory two-choice behavior assay. The concentration difference is shown by the gap between different bars on the x-axis. Mean (SE) of repency index with the same letter are not significantly different at F-value range 1.27–49.92; P-value < 0.001

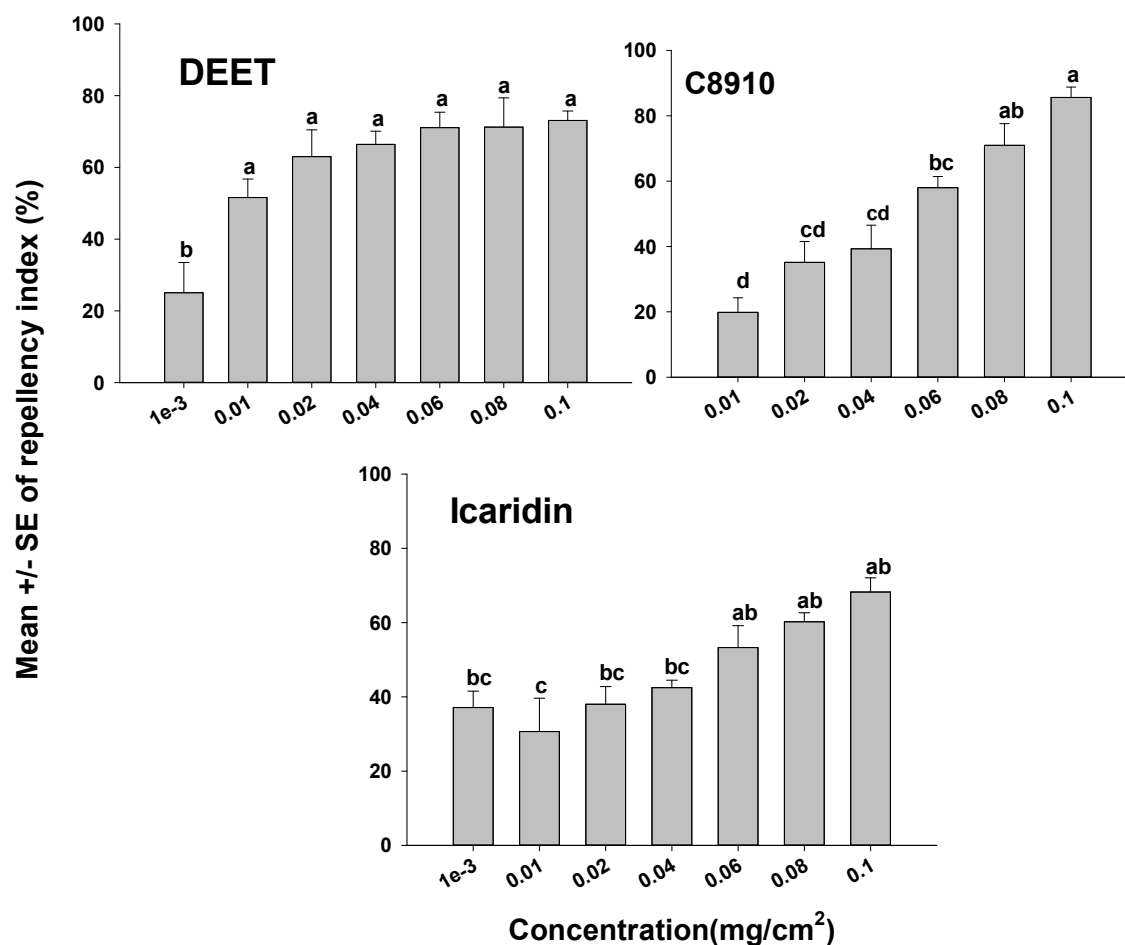


Figure 2.2. Orientation response of *T. putrescentiae* on glass surfaces treated with synthetic repellents in a laboratory two-choice behavioral assay. ANOVA determined that there was significant variation among concentrations for each of the compounds tested (F-value ranged from 6.12 to 19.25 and p-value ranging from <0.01 to 0.03). Mean ($\pm$ SE; n = 5) RIs for a given repellent with the same letter are not significantly different (REGWQ; $p > 0.05$).

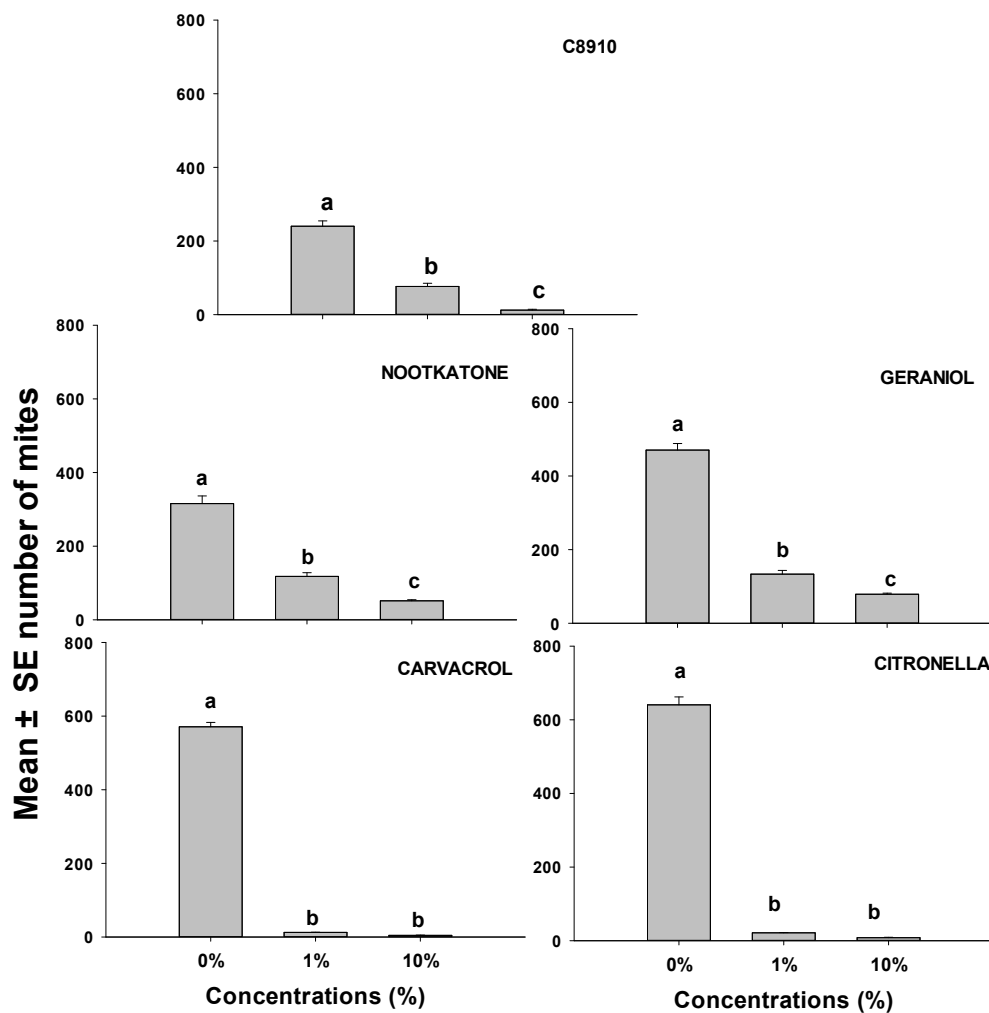


Figure 2.3. Mean ($\pm$ SE; $n = 5$) number of mobile *T. putrescentiae* progeny 14 days after inoculation of 20 mites on small dry-cured ham cubes treated with either solvent only or one of five different repellents at two concentrations. Means with the same letter are not significantly different at $p > 0.05$ using REGWQ and with ANOVA F-values of 7.29–12.16; $p < 0.01$.

Chapter 3 - Efficacy of Residual Acaricides against *Tyrophagus putrescentiae*, (Schrank) (Sarcoptiformes:Acaridae). A serious Pest of High-Valued Stored Products.

Introduction

The mold mite, *Tyrophagus putrescentiae*, (Schrank) (Sarcoptiformes: Acaridae), also known as the ham mite or cheese mite, is a serious pest of high-value durable commodities (Freitag and Kells 2013, Spieksma 1997). Infestations by *T. putrescentiae* occur throughout the food value chain, from the production stage, to processing and marketing stages, and then to consumers' homes (Brazis et al. 2008, Canfield and Wrenn 2010 and Gill et al. 2011). Mold mites infest various stored product commodities and render them valueless for sale or consumption (Brazis et al. 2008). *T. putrescentiae* infests stored grains with high moisture content (Hughes 1976, Erban et al. 2015). Mushrooms (Okabe 1999), and fungal laboratory cultures (Duek et al. 2001) have also been infested by mold mites. Likewise, any food that is rich in protein and fat content such as dry-cured hams, aged cheeses, legumes and nuts, and semi-moist high-value pet foods are susceptible to mold mite infestations (Robertson 1952, Arnau and Guerro 1994, Rentfrow 2006, Brazis et al. 2008, Erban et al. 2015).

Integrated pest management (IPM) includes prevention, monitoring, decision-making, and control of a target pest (Campbell et al. 2021). Different management tools have been researched recently for their efficacy at controlling mold mites. Some of these management methods could include detection and monitoring mite populations with traps, use of food-safe compounds and essential oils to keep mites off products, use of residual pesticides when legally

allowed, and either controlled atmosphere, freezing or heating temperatures, or fumigation to control large populations (Abbar et al. 2016, Zhao et al. 2016, Amoah et al. 2016 and Manu et al. 2021).

Pesticides are commonly used to control pest mite species in production agriculture (Guedes et al. 2016), but little pesticide work has been done with stored product mites. Research has been conducted on acaricides and natural enemies in the control of the two-spotted mite, *Tetranychus urticae* Koch (Neto et al. 2020). The population of *Varroa destructor*, the mite that infests honey bees, can be kept below an economic threshold level for up to 14 days by using one treated strip each of flumethrin, fluvalinate, and oxalic acid in each hive with ten frames (Sajid et al. 2020). From literature, it can be deduced that some residual acaricides have been available for the control of field mites, but few to no acaricides have been registered for use, at least in the USA, to control mite pests of durable stored food products (Colloff 2009). Acaricides can be highly toxic to synanthropic house dust mites, for instance *Dermatophagoides pteronyssinus* and *D. farina*, but minimal research has been conducted on using acaricides to control *Tyrophagus putrescentiae* infestations (Stara' et al. 2011a). However, Stara' et al., (2011b) reported that a combination of benzyl-benzoate, permethrin, and pyriproxyfen was active against storage mites, and Abbar et al. (2016) determined that the pesticide chlophenapyr was very effective against *T. putrescentiae*.

Dry-cured hams may become infested with large numbers of mites after hanging on racks in an aging room for three months or longer (Garcia 2004, Rentfrow et al. 2006, and Campbell et al. 2020). Since mites are very small in size, there are many places in a building that may serve as a habitat until populations reach high levels such that mites are frequently and easily seen by the naked eye at peripheral areas, shelves, ham racks and directly on aging hams. Mites can

reach hams aging on wooden or metal racks by crawling from the periphery of a room, up the legs of the rack and onto the hams (Garcia 2004). To prevent mites from moving in between shelves and floors, there is the need to treat these surfaces. Information is therefore needed on the activity of residual acaricides that are applied to different surfaces such as wood, metal, and concrete. This will cause mite mortality as they move from one surface to another in the aging room, and likely slow population growth by preventing access to large supplies of food sources (Freitag and Kells 2013).

The main objective of this work was to evaluate registered acaricides that are available, approved and used for mite pests in horticultural, field crops, veterinary medicine, and/or animal husbandry to control the mite pest of stored products, *T. putrescentiae*. A preliminary screening of various acaricides with different active ingredients was conducted to ascertain their effectiveness at killing mobile stages of mites. Acaricides with good activity for mortality of mites were further studied for their efficacy on different surfaces to quantify how long after application that the acaricides remain active.

Materials and Methods

Acaricides Screened for Activity

Some newly identified and commercially available acaricides that represented different chemical groups were screened for their effectiveness at controlling mold mites. This experiment evaluated chemicals that are either commercial formulations of products available in the USA and approved by the Environmental Protection Agency, EPA (Table 3.1), or pure compounds from different manufacturers (Table 3.2) that have the potential for registration to control mites. The acaricides that were used were all permitted for use in various ways under

regulatory agencies and represented most chemical classes of the pesticides described in the Table 3.1 and 3.2 below.

Screening Experiments

Screenings of the various chemicals were performed to determine their toxicity to *Tyrophagus putrescentiae* and suitability for further studies on residual activity over time. Mites used in these experiments were from my laboratory colony that was maintained as described in Chapter 2, p. 33–34. A stock solution of 5 mg of each acaricide in 1 ml of solvent was prepared before the start of the experiment. Concentrations of 0.001, 0.01 and 0.05 mg/cm² were then dissolved in the respective solvent (mostly acetone) with the solvent as the control treatment. With regards to commercial products, the recommended label rate was initially used for each chemical, and further work with double the recommended rates were conducted to determine the level of acaricide that was toxic to mold mites. The design for this experiment was completely randomized based on methods by Abbar et al. (2016), with five experimental units and replications for each treatment/concentration. An acetone solution of 50 µl was made for each acaricide according to the recommended rate and was placed in a glass vial (Fisher Scientific, Pittsburg-USA) with an inside surface area of 11.80 cm². The vials were rotated and turned to distribute the acaricide content on the entire surface of the vial using the Boekel Scientific 260650 tube rotator, 100-240V, manufactured by Cole-Parmer, an analytical scientific company in Vernon Hill, IL- USA. Rotations were for 1 h to dry the solvent and leave a film of the test acaricides on the inside surfaces of the vials. The rotation was meant to evenly spread the test compound on the inside vial surface so that each mite walking on the glass surface would receive a uniform contact with the toxin. Twenty mixed sexed large adult mobile mites were transferred into each coated vial and each non-ventilated lid was screwed tightly on each vial. The

experimental vials with mites were stored for 24 h at 25 °C. Mites were then transferred into clean vials with a 125 mm³ cube of ham and wet filter paper of size 25 mm² to provide humidity. The vials were tightly closed with clean cut mite mesh (Water-Resistant nylon mesh, 193 x 193” mesh, 0031” opening 40” width; Component Supply, Fort Meade, FL-US) to prevent mites from escaping from vials and a perforated vial lid was used for ventilation. Vials were then placed in a desiccator with a salt solution to maintain a high RH and appropriate temperature for 72 h as a recovery period. The desiccators contained data loggers (Onset HOBO UX100 Indoor temperature and relative humidity data loggers; Bourne-MA, USA) for recording the temperature and relative humidity, which were 25.2 ± 2 °C and 75 ± 5 %, respectively. Mite mortality was assessed by using a single brush tip. A mite that did not move after contact with the brush tip was considered dead, and mites that moved were considered alive.

Residual activity on different surfaces

This method is similar to that used by Abbar et al. (2016). After completion of the screening studies, the most effective acaricides were evaluated for their residual activity over time using disposable plastic Petri dishes (3cm diameter by 1 cm height, Fisher, Pittsburg, PA-USA) with a total inside surface area of 20 cm². The four most toxic acaricides against mold mites in the first experiments, which included amitraz, acequinocyl, pyrethrins + piperonyl butoxide and cyfluthrin, were chosen for further investigation. Concrete, wood, and metal surfaces were treated with the recommended label rate of each acaricide both from commercial or pure compounds with distilled water or acetone as the controls (n=5) and left exposed from day 1 and then 1, 2, 4, 6, 8 weeks at 25 °C, 40 % RH in total darkness.

Wood, metal, and concrete surfaces were sealed onto the floors of the Petri dishes following methods used by Arthur et al. (2008) and Abbar et al. (2016). The wood 3.1 mm thick

plywood cut into circular pieces- that filled the interior of the Petri dish (Home Depot, KS). The metal floor was made out of a zinc-plated compression washer (Home Depot, Manhattan- KS), which was fitted with non-toxic glue (Elmer's Products, Inc., Westerville-OH) on the bottom and hot glue on the sides of the dish and the holes inside the washer (Adhesive Technologies, Inc. Hampton-NH) were used to seal inside the dish to prevent mites from hiding under the washer and wood, and thus maintain mites in contact with these surfaces. 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 2.5 mm in height by using a spatula to flatten the moist concrete. The dishes containing the concrete were then tapped several times on the floor to remove all air bubbles from the thick solution. The three surfaces were prepared 2 weeks prior to the application of the pesticides. One hundred ml of the recommended label rates of chemicals were formulated in a glass beaker using distilled water or acetone as a diluent for commercial and pure compounds, respectively. Solutions were applied on the individual surfaces using an artists' airbrush (Badger 100, Badger Corporation, Franklin Park, IL). The volume of spray was 180 μ l applied to all inside surfaces of each dish. Distilled water or acetone was sprayed at 180 μ l to serve as a control. Five experimental replicates were prepared and tested for each acaricide and control treatment for each of the three surfaces. The dishes were held in storage cabinets under ambient conditions (approximately 25 °C and 70 % R.H.) until they were used for the mortality bioassays at either one day or 1, 2, 4, 6, or 8 weeks after treatment.

Twenty unsexed large adult mobile mites were transferred to each surface for 24 h at the beginning of each test period. After the 24-h exposure time, mites from each dish were

transferred to a clean Petri dish with a small piece of wet filter paper (25 mm²). Mortality was assessed following a recovery period as described for the screening experiments.

A completely randomized design with 5 x 6 factorial structure of 5 different treatments including four acaricides and a control and 6 post-application periods for each of the three surfaces in these experiments were conducted.

Data Analysis

Data were analyzed using the PROC GLIMMIX procedure in SAS version 9.4 (SAS Institute Inc. 2010). The data were adjusted using the arcsine transformation (Motseller and Youtz 1961) of the proportion that was killed by the chemical. It was then submitted separately for screening experiments and aged surface experiments to one-way and two-way ANOVAs using GLM models, respectively. The type of chemical and time after application and their interactions were compared to determine the effect on mite toxicity for each of the three surfaces. Treatment means separation was done using Tukey's Honest Significant Difference (HSD) test at the 0.05 significance level.

Results

Screening Bioassays

Pyrethrin + piperonylbutoxide, etofenprox + piperonylbutoxide, and cyfluthrin, were toxic at the label rate and double the label rate for surface treatment. (Table 3.3). Treatment with the recommended rates of cyfluthrin, etofenprox + piperonylbutoxide and pyrethrin + piperonylbutoxide had percent mortalities of 92.5 ± 0.7 , 81.0 ± 0.7 and 82.0 ± 1.4 , respectively. In contrast, methoprene and novaluron + pyriproxyfen, which are both growth regulators, were the least effective with 17.5 ± 5.6 and 25.0 ± 1.8 % mortality when the recommended label rates were applied, and only 32.5 ± 3.7 and 42.5 ± 1.6 % mortality when double the recommended

rates were applied (Table 3.3). In our preliminary work, pure methoprene was diluted for an application of 0.05 mg/cm² applied to glass onto which mites were walking for 24 h, and I found it caused 1.6 ± 0.7 % mortality. The mortality of mites increased very little when I doubled the recommended rate of the commercial formulation (Table 3.3).

With regards to the pure compounds, the highest concentration tested was 0.05 mg/cm² at which most of the chemicals were highly effective at killing mites (Table 3.4). Acequinocyl and amitraz were the most effective amongst all five compounds, with mortality percentages of 98.7 ± 2.6 % and 97.5 ± 5.0 % respectively. Clofentizine, diflubenzuron, etoxazole, flumenthrin and hexathiol had mortality rates below 90 % of 0.05 mg/cm² and therefore were not included in the subsequent residual surface treatment experiments (Table 3.4).

Residual Activities on Different Surfaces

The main effects of time and acaricide as well as their interaction effect (time x chemical) were significant at $P < 0.05$ for all three surfaces (Table 3.5). Amongst the four compounds that were tested, amitraz was the most efficacious at killing mites throughout the weeks of exposure. Acequinocyl and amitraz retained their potency, which is evidenced by percent mortality of greater than 50 % on all surfaces throughout eight weeks. These two compounds had the highest percent mortality on metal with both achieving a 100 % kill on day one. Percent mortality for these two chemicals was variable over time. With regards to mean percent mortality with time, efficacy of the tested chemicals reduced with time. With the exception of pyrethrin + piperonyl butoxide which was approximately 40 % mortality even after day one of application on all surfaces, the other three chemicals significantly reduced mite numbers.

Acequinocyl percent mortality ranged from 97.0 ± 0.5 % to 90.0 ± 1.4 % from day 1 to week 2 of post application, but on concrete reduced drastically from four to eight weeks after

application (69.0 ± 1.8 % to 35.0 ± 1.4 %). Similar trend was recorded for acequinocyl on wood surfaces with highest mortality at 93.0 ± 0.2 % one day post application and the lowest mortality recorded at 51 ± 1.8 % on week eight. On metal, 100 % mortality was recorded a day after application, and although % mortality decreased with time it was still 78.2 ± 2 % by week eight which indicates, this chemical works well on metal surfaces.

Amitraz had significantly higher percent mortality of 90–100 % effective one day, one week, and 4 weeks after treatment than all the chemicals tested. Amitraz had 100 % percent mortality on both concrete and metal after a day of application. Amitraz mortality ranged from 100.0 to 68.0 ± 0.8 % from day one to week eight on concrete, 98.0 ± 0.5 to 80.0 ± 1.6 % on wood and 100.0 to 85.0 ± 1.6 % on metal respectively. Its potency declined with time post-treatment on all surfaces. However, it was still within the range of 70–80 % on week eight. Amitraz was therefore the most effective acaricide amongst the four chemicals tested for residual activity (Table 3.6). This indicates that amitraz could potentially be applied every 4 weeks to control mites.

Cyfluthrin was effective initially at one the day and one-week post-application with percent mortality of 85.0 ± 0.1 and 75.0 ± 0.1 on concrete, 91.0 ± 0.6 and 96.0 ± 0.8 on wood and 90.6 ± 0.6 and 91.0 ± 0.4 , respectively. However, there was a significant reduction in % mortality for the rest of the longer post-application periods. The percent mortality post application recorded for cyfluthrin in week 8 were 8.7 ± 0.2 %, 11.2 ± 0.7 % and 9.6 ± 0.2 % on concrete, wood and metal respectively. Pyrethrins + piperonyl butoxide was the least effective at day one 49.0 ± 2.3 %, 44.0 ± 1.8 % and 40.0 ± 1.0 % mortality for concrete, wood and metal respectively. Pyrethrins + piperonyl butoxide had a significant decrease in percent mortality across all weeks and on the three different surfaces. Just as cyfluthrin, pyrethrin + piperonyl

butoxide did not exceed 50 % mortality, however, a higher mortality around 40 % was recorded throughout the week and on the different surfaces for the first day. There was an exceptional higher mortality percentage of 57.4 ± 1.8 on metal in week one but drastically reduced in mortality at other times. Lastly, cyfluthrin had impressive percent mortality for the day one and week one of residual surface treatment making about 90.0 % mortality on all surfaces, but its potency reduced substantially afterwards. Pyrethrin + piperonyl butoxide performed the worst amongst all the chemicals tested. It recorded the lowest percent mortality of 4.8 ± 0.3 % on metal on the eighth week (Table 3.6).

Discussion

Several studies have looked into the use of pesticides to control stored product pests and prevent postharvest losses, but minimal information is available on the protection of dry-cured ham from mites during aging (Arthur 2008, Athanassiou et al. 2009, Collins 2006, Freitag and Kells 2013, Abbar et al. 2016). Many pesticides are not labeled for food contact surfaces which could prevent their use in food processing areas with food contact surfaces (Arthur et al. 2009). However, some pesticides can be applied to surfaces that do not come in contact with food and have extended residual effect which is advantageous because there is a reduced number of times that these chemicals need to be applied to the surfaces in commercial food storage rooms (Arthur 2008, Arthur et al. 2009, Athanassiou et al. 2011, Wijayaratne et al. 2012, Athanassiou et al. 2014).

Chlophenapyr was very effective at controlling mold mites, even 8 weeks post-application (Abbar et al. 2016). Although mold mites are acarids, very few studies have evaluated acaricides on this stored product pest. Freitag and Kells (2013) conducted research on the residual effects of some acaricides for suppressing *T. putrescentiae* for 24 to 48 hours on

nonporous surfaces to serve as a protective barrier near susceptible food packages. They found that increasing humidity reduced acaricide efficacy and that the use of adjuvants had no effect on acaricide efficacy, but the type of application either wet, slurry or dry acaricides significantly affected efficacy. In contrast, our study evaluated the residual effects of acaricides up to eight weeks after application by using one type of application which is wet form.

In our research, twelve chemicals were screened, with only four selected for further study in residual treatment experiments. Cyfluthrin and pyrethrin + piperonylbutoxide were very effective when the recommended rates of these commercial products were used. Percent mortalities were 100.0 ± 0.0 and 95.8 ± 0.9 % respectively when double the recommended rate were used. Methoprene and novaluron + pyriproxyfen were very ineffective both at the recommended rates and double the recommended rates were applied. Hence, they were not used for the surface treatment (Table 3.3).

Methoprene was ineffective at controlling mold mites during screening. Methoprene is a juvenile hormone analog that is effective against larval stages of most insect pests. However, it was ineffective at controlling mold mites even at double the recommended rates against adult mites (Silberhorn 2005, Arthur and Fontenot 2012). Although it is not effective compared to the other chemicals used, a few mites died during the screening. This suggests that mites might have died due to natural death or accidental death from the experiment but not necessarily from the effect of the methoprene as it was in the case of the control. Because only adult stage mites were used in our study, it was not expected that there would be significant mortality given that insect growth regulators have no effect on mite immatures during molting. Future work should look at the effect of methoprene on the mortality of immature mites. Sutton et al. (2011) reported 100 % suppression of exposed *T. castaneum* larvae during a 16 wk residual experimental period in

outdoor trials when different packaging surfaces were treated with methoprene which suggests methoprene might be effective for other life stages of mites but not the adult mobile mites. Methoprene had a very high efficacy as an aerosol when *T. castaneum* larvae were treated by slowing down their growth to adulthood (Arthur 2012).

Novaluron + pyriproxyfen was not used for the residual experiment due to its low efficacy in the screening study. Novaluron + pyriproxyfen is a growth regulator that is known to effectively reduce acarine populations (Showler et al. 2019). These researchers reported that novaluron + pyriproxyfen halted the larval growth of *Amblyoma americanum* affecting metabolic function, which prevented the larvae from molting into adults on calves. In a study to control *Aedes aegypti* with pyriproxyfen, spinosad, and novaluron, a chitin synthesis inhibitor, was more effective at killing mosquito larva than pyriproxyfen (Marina et al. 2020). However, the combination of these treatments in the current study were less effective at controlling adult mites. Combining novaluron + pyriproxyfen with the pyrethroid, permethrin, caused greater mortality of all mite species (Stara' et al. 2011b). This suggests that acaricides that function as growth regulators may need to be combined with one or more pesticides that are persistent and long-lasting on surfaces. Same with methoprene, because adult stages were used in our studies using immature stages of mold mites are needed to verify the effectiveness of these growth regulators against mites. Since in our experiments I used already matured adult mites, novaluron effects were not as I expected. In future, the mites after coming in contact with the treated surfaces could be allowed to mate and the eggs hatched and monitored to see if the growth regulators affect their hatchability of the eggs and growth of the larva to other life stages.

Of all the pesticides tested; amitraz, acequinocyl, cyfluthrin, and pyrethrin + piperonyl butoxide were the most effective, with greater than 90 % mortality during the screening test

against mold mites, which led to their selection for evaluation in subsequent studies to determine their effectiveness against mold mites on different surfaces. Mite control was consistent for amitraz until week eight on all surfaces. Abbar et al. (2016) also found a consistent and persistent chemical in chlophenapyr which gave a 100 % kill of mites throughout the experiments on different surfaces over time. Although, amitraz was not 100 % effective on all surfaces and across all weeks, it was the most promising chemical among the four. Amitraz results from this study were similar to those of Garris and George (1985) against *Boophilus microplus*. Amitraz is a broad-spectrum insecticide or acaricide a rapid control action on ecto-parasites such as mites, and ticks, and can persist enough to control all life stages of these parasites (Gutpa 2007). Amitraz is a formamide antiparasitic agent that works by interfering with the nervous system of these pests (Richardson 2013). Amitraz is also effective at controlling *Varroa* mites in honey beehives (Rinkevich et al. 2017, Pohorecka et al. 2018, and Sajid et al. 2020).

Acequinocyl was highly toxic to *T. putrescentiae* for the first three to four weeks after treatment, but its efficacy declined starting from 3 to 4 weeks, depending on the surface to which it was applied. Similarly, Puchalska and Piotrowska (2016) reported 100% mortality of *Typhlodermus pyri* females after initial contact with acequinocyl three days after it was sprayed on leaves. Acequinocyl is a contact acaricide that kills all life stages of various mite species including predatory mites such as *Amblyseius swirskii*, *Amblyseius cucumeris*, *Neoseiulus californicus*, and *Phytoseiulus persimilis* with residual effects lasting over 28 days (Chong et al. 2017). Acequinocyl is a pro-insecticide that must be deacetylated before it is active. It acts as an inhibitor in the mitochondrial electron transport complex III, which prevents energy transport (BASF 2013).

Piperonyl butoxide is an insecticide synergist which aids in the potency of pyrethroids and carbamates with respect to controlling pests. (Tozzi 1999). Pyrethrin degrades faster after exposure to surfaces due to temperature and UV light changes (Sutton 2009). I hypothesized that pyrethrin + PBO application would result in high mortality levels on all surfaces across weeks because of lack of exposure to UV light. However, pyrethrin + piperonyl butoxide caused less mortality on all surfaces than the other treatments (Table 3.6). This indicates that piperonyl butoxide did not work synergistically with pyrethrin to control mold mites at the tested rates since PBO effects is much based on concentrations.

Cyfluthrin is a residual pyrethroid insecticide for surface treatment in milling and processing plants to control insects (Zettler and Arthur 2000). Cyfluthrin was 92.5 ± 6.5 % effective at controlling mites during the screening process. However, its residual effectiveness lasted for only 1-2 weeks, which prevents it from being used to control mites in the dry-cured ham industry (Table 3.6). Arthur (1999) reported that, temperature changes and chemical age could affect the effectiveness of cyfluthrin, which may account for the lower mortality of mites over time.

The majority of chemicals were more effective on metal over time, when compared to concrete and wood. Activity of amitraz and acequinocyl persisted on the different surfaces for the entire period of the study. The wood in this study was not painted to mimic the ham aging room. The uneven and porous nature of wood has the potential to hold chemicals for longer time than other materials (Arthur et al. 2009). Metal does not absorb insecticide so more remains on the surface where mites can come in contact. Wood and concrete can absorb insecticide below the surface making it less available to the mites. Therefore, surface material must be considered in stored product pest management if efficacy information is available. Amitraz was effective at

controlling mites and persisted on wood, metal and concrete for the longest time in our studies at 4 to 6 weeks for metal and 6 to 8 weeks for concrete and wood. If amitraz or acequinocyl were sprayed on surfaces, reapplication would need to occur sometime between 4 and 8 weeks to control mite growth.

Most of the acaricides screened have been approved by the EPA for either agricultural, horticultural, or veterinary medicine use to control mites and other ectoparasites. For instance, etoxazole, hexythiazox and acequinocyl are acaricides for controlling mites on beets sugar roots, cotton, vegetables, field corn, ornamental plants, tropical and subtropical small fruits, pome fruits etc. (EPA 2018, EPA 2022a, 2022b). Clofentezine is known to control mites such as the two-spotted spider mite, European red mite, pacific spider mite, yellow spider mite, and the McDaniel spider mite, all of which are related to agriculture and horticulture but not for stored product mites (EPA 2016). Other insecticides such as pyrethrin + PBO are used on surfaces in areas such as receiving, sorting, packing, cracks, and crevices in food processing environments to control insects and mites (EPA 2013). Amitraz is used in horticulture, agriculture, and animal husbandry to control ectoparasites such as mites, fleas, lice, ticks etc. (EPA 2002, Gutpa 2007). It has been recently registered to control *Varroa* mites in honeybee hives (EPA 2022c). Amitraz was the most effective compound at controlling mold mites on surfaces over time. It could therefore be developed and included as a tool for IPM of storage mites, including *T. putrescentiae*. However, future work would need to be conducted to investigate how safe amitraz is when it is used around stored food products for mite control through residue testing in foods and on food contact surfaces after the label is explained to include this application on food and human safety issues with this compound is discussed.

T. putrescentiae infest dry-cured hams in aging rooms. Applying chemicals to surfaces that can stay on those surfaces for a long period of time can serve as a tool for preventing or slowing mite infestations since mites are crawlers and they need to walk on surfaces to get to the ham. Inclusion of the acaricides that were effective at killing mites in the integrated pest management program will reduce mite populations. Both growth regulators in addition to other pesticides take care of mites in all their life stages. With the objective of this study being to find selected acaricides that can be used as a residual treatment to reduce the mite population, amitraz and acequinocyl could be adopted as a preventive measure to control mold mites in an aging room, pending approval from EPA and USDA-FSIS for this specific application.

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Table 3.1. General information for registered commercial pesticides used for screening for the control of *Tyrophagus putrescentiae*.

Chemical Group	Active Ingredient	Trade	% Active ingredient	Applied label rate	Manufacture
		Name	in stock solution	active ingredient (per m ²)	
Benzylureas	Novalrun + Pyriproxyfen	Tekko [®]	1.3 + 1.3	1.00 µl	Control Solutions; Illinois, US
Insect Growth Regulator	Methoprene	Diacon [®]	33.60	0.051 µl	Wellmark International; Illinois, US
Pyrethroids US	Cyfluthrin	Cykick [®]	6.00	1.50 µl	BASF Corporation; Florida,
Pyrethroids US	Pyrethrins + Piperonyl butoxide	ULD-BP [®]	1.0 + 5.0	1.51 µl	BASF Corporation; Florida,
Synthetic Pyrethroids	Etofenprox + Piperonyl butoxide	ZenproX [®]	16.2 + 64.8	3.00 µl	Wellmark International Illinois, US

Table 3.2. General information on active ingredients for commercial acaricides tested for their efficacy at controlling *T. putrescentiae**.

Chemical group	Active Ingredients	Trade Name	% Purity	Applied label rate active Ingredient (per m ²)	Commercial Registrant
Benzylurea	Diiflubeznuron	Dimilin Adept [®]	98.0	0.05mg	Arysta Lifescience, Cary - NC
Chitin Synthesis Inhibitors	Etoxazole	TetraSan [®]	98.0	0.05mg	Valent, Walnut Creek - CA
Insect Growth Regulators	Clofentezine	Ovation SC [®]	99.8	0.05mg	Makhteshim Agan, Raleigh - NC
Pyrethroids	Flumethrin	BAN [®]	> 96.1	0.05mg	Bayer, Shawnee Missions -KS
Quinoline	Acequinocyl	Kanemite [®]	98.2	0.05mg	Arysta Lifescience, King of Prussia- PA
Triapentadiene	Amitraz	Mitac [®]	98.0	0.05mg	Aventis CropScience, NC

*All pure active ingredients were obtained from Sigma Aldrich, St. Louis, MO, USA

Table 3.3. *T. putrescentiae* mortality after exposure to several commercially formulated acaricides at two concentrations in screening bioassay using glass vial.

Chemical	Mean % Mortality (SE)			
	Control	1x Label rate	2x Label rate	$F_{(2)}$, P value
Cyfluthrin	2.8 (0.2) c	92.5 (0.7) ab	100.0 (0.0) a	176.7, <0.01
Methoprene	2.5 (0.1) c	17.5 (1.6) b	32.5 (3.7) a	55.2, < 0.01
Novaluron + pyriproxyfen	0.0 (0.0) c	25.0 (1.8) b	42.5 (1.6) a	88.6, < 0.01
Etofenprox+ piperonylbutoxide	0.0 (0.0) b	81.0 (0.7) a	86.8 (0.5) a	136.0, < 0.01
Pyrethrin + piperonylbutoxide	0.0 (0.0) c	82.0 (1.4) b	95.8 (0.9) a	68.0, < 0.01

Means followed by the different lower-case letter for each active ingredient are not significantly different among concentration-treatments of the same chemical. Data analyses were with ANOVA followed by the HSD test at 0.05 significance level.

Table 3.4. Percent Mortality of *T. putrescentiae* that was exposed to several pure acaricide active ingredients dissolved in acetone at three concentrations.

Chemical	Mean % Mortality (SE)			
	Concentration (mg/cm ²)			
	0.0	0.001	0.01	0.05
Acequinocyl	7.7 (8.9) i	70.3 (5.8) dec	82.1 (15.2) abcd	98.7 (2.6) a
Amitraz	13.4 (4.5) i	45.9 (8.9) gh	71.9 (9.3) cd	92.6 (5.4) ab
Clofentezine	5.1 (5.9) i	45.7 (9.8) gh	70.1 (9.3) cde	77.7 (12.7) bcd
Diflubenzuron	10.6 (7.0) i	50.1 (16.9) fgh	42.7 (15.4) gh	77.2 (9.5) bcd
Etoxazole	7.4 (2.1) i	51.9 (21.5) fg	39.9 (30.3) fg	83.4 (18.4) abc
Flumenthrin	4.8 (2.1) i	33.3 (14.3) h	53.1(14.1) efg	88.5 (5.0) b
Hexythiazox	12.8 (10.3) i	48.6 (9.4) fgh	64.8 (9.2) cde	62.8 (9.2) def

Means followed by the different lower-case letter for each active ingredient are not significantly different among concentration-treatments of the same chemical. Data analyses were with ANOVA followed by the HSD test at 0.05 significance level.

Table 3.5. ANOVA for main effects residual exposure time and acaricide on concrete, metal, and wood to *T. putrescentiae* mortality.

Surface	Source	df	<i>F</i>	<i>P-value</i>
Concrete	Time	5	84.85	<.0001
	Acaricide	4	594.30	<.0001
	Time x acaricide	20	18.98	<.0001
iMetal	Time	5	145.27	<.0001
	Acaricide	4	1609.19	<.0001
	Time x acaricide	20	36.79	<.0001
Wood	Time	5	173.09	<.0001
	Acaricide	4	1279.96	<.0001
	Time x acaricide	20	38.67	<.0001

Table 3.6. Residual activity of acequinocyl, amitraz, cyfluthrin and pyrethrins +piperonyl butoxide on the mortality of *T. putrescentiae* after exposure for 24 h for up to 8 weeks after application to wood, metal and concrete surfaces.

Surface	Pesticides	Mean % Mortality (SE) per time period after application						
		1 Day	1 Week	2 Weeks	4 Weeks	6 Weeks	8 Weeks	
Concrete								F ₍₅₎ , <i>P</i> val
	Control	4.0 (0.8) Ad	0.0 (0.0) Bc	4.0 (0.8) Ac	1.9 (0.5) ABd	2.7 (0.5) ABd	4.0 (0.8) Ac	1.4, 0.3
	Acequinocyl	97.0 (0.5) Aa	93.6 (0.2) Aa	90.0 (1.4) Aa	69.0 (1.8) Bb	48.8 (2.5) Cb	35.0 (1.4) Db	57.9, < 0.01
	Amitraz	100.0 (0.0) Aa	85.0 (1.8) Bb	94.0 (0.8) Aa	94.0 (0.8) Aa	89.0 (0.8) Ba	68.0 (0.8) Ca	5.1, < 0.01
	Pyrethrins + Piperonyl butoxide	49.0 (2.3) Ac	13.6 (0.9) Bc	9.3 (0.1) BCc	7.6 (0.5) BCd	6.7 (0.2) Cd	5.6 (0.1) Cc	54.0, < 0.01
	Cyfluthrin	85.0 (0.1) Ab	75.0 (0.1) Bab	49.4 (0.5) Cb	38.0 (1.1) Dc	23.0 (1.1) Ec	8.7 (0.2) Fc	63.7, < 0.01
	F ₍₄₎ , <i>p</i>	266.4, < 0.01	33.7, < 0.01	556.3, < 0.01	273.8, < 0.01	148.2, < 0.01	225.2, < 0.01	
Wood								
	Control	2.5 (0.4) Bd	0.9 (0.4) Bc	3.5 (0.4) Bc	14.0 (1.5) Ad	2.5 (0.5) Bd	2.9 (0.9) Bd	7.8, 0.01
	Acequinocyl	93.6 (0.2) Aab	93.9 (0.2) Aa	91.0 (0.9) Aba	83.0 (1.3) Bb	59.0 (2.3) Cb	51.0 (1.8) Cb	38.9, < 0.01
	Amitraz	98.0 (0.5) Aa	92.0 (0.9) Ba	91.8 (0.6) Ba	91.6 (0.6) Ba	90.2 (0.7) Ba	80.0 (1.6) Ca	8.4, 0.01
	Pyrethrins + Piperonyl butoxide	44.0 (1.8) Ac	44.0 (1.5) Ab	7.7 (0.3) Bc	8.0 (0.1) Bd	6.6 (0.3) Bd	4.0 (0.2) Bcd	68.3, < 0.01
	Cyfluthrin	91.0 (0.6) Ab	96.0 (0.8) Aa	50.6 (2.3) Bb	38.0 (1.9) Cc	16.2 (1.4) Dc	11.2 (0.7) Dc	126.2, < 0.01
	F ₍₄₎ , <i>P</i>	430.6, < 0.01	362.7, < 0.01	286.7, < 0.01	181.5, < 0.01	180.3, < 0.01	168.0, < 0.01	
Metal								
	Control	3.1 (0.4) ABd	0.0 (0.0) Bc	3.8 (0.5) ABc	2.5 (0.7) ABc	2.7 (0.5) ABd	6.4 (1.0) Ab	2.4, 0.07
	Acequinocyl	100.0 (0.0) Aa	94.0 (0.8) ABa	88.0 (0.9) Ba	89.6 (0.9) Ba	77.2 (1.2) Cb	78.0 (1.3) Ca	17.1, < 0.01
	Amitraz	100.0 (0.0)Aa	95.0 (0.1) ABa	88.0 (0.9) BCa	91.0 (1.3) BCa	91.0 (1.5) BCa	85.0 (1.6) Ca	4.6, 0.04
	Pyrethrins + Piperonyl butoxide	40.0 (1.0) Bc	57.4 (1.8) Ab	7.9 (0.5) Cc	8.6 (0.2) Cc	5.6 (0.2) Cd	4.8 (0.3) Cb	130.2, < 0.01

Cyfluthrin	90.6 (0.6) Ab	91.0 (0.4) Aa	53.8 (2.5) Bb	45.4 (1.7) Bb	28.2 (2.3) Cc	9.6 (0.2) Db	85.5, < 0.01
$F_{(4)}, P$	1339.0, < 0.01	411.5, < 0.01	203.2, < 0.01	300.0, < 0.01	182.3, < 0.01	288.8, < 0.01	

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 for each active ingredient are not significantly different. Data analyses were with ANOVA followed by the HSD test at 0.05 significance level.

Chapter 4 - Physical Barriers to Prevent Infestation by *Tyrophagus putrescentiae* (Sarcoptiformes: Acaridae) on dry-cured Hams on Aging Racks

Introduction

The ham mite, *Tyrophagus putrescentiae*, is one of the most economically important pests of durable stored food commodities, especially foods with high protein, high fat, and high moisture contents (Erban et al. 2015, Abir Sulaiman Al-Nasser 2017). A typical American dry-cured ham is processed with a mixture of salt and one or more optional additives. After curing and refrigeration for 40 or more days, hams are then stored in a room-temperature aging room for up to two years to attain the desirable characteristics under a favorable environmental condition of 24–35 °C and 55–65% relative humidity (USDA 1999). Unfortunately, the growth and development of *T. putrescentiae* is enhanced by these conditions (Graham et al. 2011). Dry-cured hams are hung for prolonged lengths of time in an aging space where they become infested with mites. However, for the desired flavor and taste to meet the demands of the market, dry-cured hams are hung for periods up to two years (Toldrá et al. 1997). Mites can infest ham within the short period of three months (Rentfrow et al. 2006), but longer ham aging period increases the risk of substantial infestation. Long-aged hams have a more complex and intense flavor that is appreciated by most consumers (Marriott and Schilling 2004) resulting in a high-priced specialty food product. The aging results in the loss of moisture, and increased proteolysis and lipolysis, which give it the unique flavor and taste (Toldrá et al. 1997).

Ham mites can be found along all the food value chain from storage of raw materials through to processing and distribution, including freshly harvested grains, nuts and fruits their processed value-added finished product for consumers at time of sale (USDA 2016). The predominant control method for stored product mite has been fumigation with toxic gas. Methyl bromide is an exceptional fumigant pesticide that is used to control ham mites but it is no longer allowed in the USA for most agricultural uses due to its status as an atmospheric ozone-depleting substance. Research like this reported here represents the investigation of various other control methods as methyl bromide alternatives (EPA 2008, EPA 2014). Research projects has been performed with different experimental treatments to explore alternatives to methyl bromide as potential applications for controlling mites (Phillips et al. 2008, Zhao et al. 2012, Abbar et al. 2016).

Monitoring mite populations by different sampling methods and using trap captures to estimate relative pest density or infestation level relative to their action threshold is necessary to determine treatment choice to control mites (Hutchins 1994). In a study by Amoah et al. (2017), it was confirmed that the number of ham mites found in traps were linearly related to the mite density in a given area at a given time. Early detection and estimating pest density gives information on infestation over time and space, which helps with management decisions (Savoldelli 2006). It has been documented that in order to monitor ham mites, traps are very important for sampling and estimating the relative number of mites over time and space (Amoah et al. 2016 and 2017).

It was reported that mite population decrease substantially after fumigation is applied. However, mite populations and their activities increase in a matter of weeks after in the ham aging rooms after fumigation (Amoah et al. 2016). Mites are very prolific and small in size.

Hence, they can find refuge in a variety of places until they reach high infestation levels in the shelves' periphery and can get to shelves by different means with the most prevalent being crawling from the floor to the shelves (Garcia 2004).

At this date the only methyl bromide that can be used to fumigate ham facilities are the existing stocks of the gas that had been government-approved for such uses. Although methyl bromide continues to be produced, none is allowed for nearly all pre- and post-harvest agricultural uses in the USA. Alternative fumigants are being researched and tested to determine their advantages and disadvantages as methyl bromide alternatives for pest control. Integrated pest management is a good alternative to fumigation. Many different tactics that can be implemented as part of an IPM program have been researched, including monitoring, using food safe compounds, physical controls such as extreme cold and heat, and others. Natural repellents and commercial synthetic acaricides ham mites are elaborated in Chapters 2 and 3 of this dissertation. The potential physical barriers against movement and invasion of ham mites as potential components of IPM are the focus in this study described below.

Physical barriers have been successful in agriculture plant protection against several insect pests (Vincent et al. 2003). For example, the use of a micrometric net roofing for fruit production of cherries, grapes and different apple varieties showed significant reduction pest damages in both production and storage of these fresh fruits (Holtz et al. 2020). Experimental fences were found to exclude low-flying insects such as anthomyiid flies in some high-valued crops such as cole and onion (Boiteu and Vernon 2001). In a study by Vernon and MacKenzie (1998), a 90 cm high fence excluded 80 % of flying female cabbage flies, *Delia raicum* (Diptera: Anthomyiidae) in a field. In a recent study, the food grade chemical solution of 40 % Propylene Glycol + 1% Propylene Glycol Alginate + 1% Carrageenan) was incorporated into polyester

weft-knitted mesh nets to control ham mites by serving as a barrier between mites and the ham (Campbell et al. 2020; Al-Amin et al. 2021).

The objective of this study was to evaluate some liquid and sticky treatments that could serve as barriers against mites' movement toward dry-cured ham on the laboratory bench and in a simulated aging room. This research seeks to provide control measures that are less expensive and accessible to dry-cured ham producers.

Materials and Methods

Mite colonies

Colonies of mites, *T. putrescentiae*, were prepared and kept at room temperature, 25–30 °C and 70–80 % relative humidity under 24 h of darkness. The procedure for maintaining the cultures were followed from Abbar et al. (2016), and are described with more detail in Chapter 2, p. 33–34. The mites used in these experiments were taken from the rearing jars that were two-three-week-old following inoculation.

Barrier Experiment in Laboratory Experimental Arenas

Three studies were conducted in experimental benchtop arenas to study the orientation of wandering mites to a food bait protected by liquid or gel barriers. The liquids for testing were placed in a large glass 90 mm diameter Petri dish that served as a moat to protect the food from migrating mites. The Petri dish was firmly placed at the bottom center of an empty plastic rectangular shoe box with a volume of 5.7 l and a latched-lid. The shoe boxes had dimensions of 35.2 x 15.9 x 7.9 cm as L x W x H. Before the liquid treatments were placed into the large Petri dish, a double-sided tape was placed at the center of the dish and then a 20 mm diameter glass Petri dish was placed on it. The smaller dish contained the food bait, which was a 1 cm³ piece of mite rearing diet as described in Chapter 2, and small dish was placed at the center of

the 90 mm Petri dish and represented the destination of the successful dispersing mites. Each treatment was replicated five times. The liquid treatments added to the large dishes included 25 ml each of either water only, soapy water (made of 10 ml dish soap and 15 ml of water), mineral oil, vegetable oil or glycerol. The gel treatments tested were Vaseline (100% pure petroleum jelly, Walmart Inc, Bentonville, AR- USA) or Tangletrap sticky coating (Tangle-foot® tangle-trap coating, Brushable 8 oz, Marysville, Ohio-USA). The Vaseline treatment was applied top to bottom of the inside surface of the side-wall of the 90 mm Petri dish at a thickness of approximately 0.5. The treatment with the Tangletrap sticky coating was spread onto the top surface a rectangular piece of construction paper, 7 x 5 cm and attached to the center of the floor of the 90 mm dish with double-faced sticky tape. Untreated control boxes had no barrier treatment in the 90 mm dish and all else was the same with a food-bait placed in the center of the small dish.

Mites for these experiments were added to each shoebox by using fine-tipped forceps to insert a 5 mm² piece of white paper into a 3-week-old mite colony for 60 s, and then immediately placing the paper onto the floor of a given plastic shoe box at a randomly selected corner. I counted the number of mites on six pieces of inoculated paper and found there were approximately 150 ± 5 mites of mixed sex and ages per paper that was added to each shoebox. The shoebox was then closed with the lid that was modified with a perforated opening covered with mite-proof mesh to allow for air ventilation. The inside top edge of each bowl was coated with Vaseline to deter escape of mites. All the shoeboxes were placed on shelves in randomly selected positions under total darkness and room conditions of 25° C and approximately 40% RH for 24 h. The numbers of mites that had arrived in the 20 mm Petri-dish with the food-bait in each box were then counted.

In a second experiment I exchanged the 90 mm Petri-dish with plastic dish of 95 mm diameter and 60 mm high with similar treatments. Such a dish was used in this experiment to require the responding mites to climb a tall vertical surface to reach the food bait. The same procedures were then done, and data were collected after 24 h as in the first experiment.

A third lab-bench experiment was done that included a small replica of a wooden leg of a ham-aging rack upon onto which a food bait was placed, and the wooden leg was standing in 95 mm diameter x 60 mm tall plastic container that held the liquid barrier being tested. In place of using the smaller 20 mm Petri dish for the food bait, rectangular pieces of heavy-duty plywood were cut and glued together to make small posts with a height of 75 mm and width of 20 mm square per side as the food bait holder. In this experiment, the various liquid treatments were placed in the plastic dish and, since I conducted a preliminary study and observed that wood absorbed some of these liquids, the volumes of the liquid treatments were increased to 50 ml. The same treatments and procedures were applied to this experiment, and mite numbers were counted after 24 h. Since the height of the wood with its food bait was taller than the wall of the plastic shoebox, the shoeboxes were not closed for this experiment. Petroleum jelly was applied to the top inside periphery of the shoebox in order to prevent mites from escaping. Mites found on the bait and the top of the wooden post were counted after 24 h. Five replicates of each of the treatments were established.

Simulated Aging Room

An insect rearing room in the Department of Entomology at Kansas State University was used as a simulated ham aging room for a series of experiments (Fig 4.1). The room had a length x breadth x height of 4.3 x 2.5 x 2.7 m with no windows and a single door at one end. A metal shelf rack (Work Choice model 5t-12; MyWorkChoice, Sandusky, OH) from which hams were

hung was placed in the room. The rack was 1.8 m tall by 0.5 m wide and had three shelves of 1.2 m length and 0.5 m width. The three rack shelves were at heights of approximately 0.3, 0.6, and 1.8 m from the floor. The rack was placed at the center of the room and 0.6 m from the walls of the room. Markings were made on the floor to indicate the placement of the rack legs. Two commercially provided dry-cured hams (Tripp Country Hams, Brownsville, TN, USA), each weighing 6–8 kg, were hung from the top shelf at approximately 0.5 m apart.

Following every 24 h replicate of these two experiments, the floor of the room was cleaned with soapy water and bleach and the entire rack and shelves were sprayed with 70 % ethanol and dried with wet clothes. Hams were wiped clean of mites with warm water, a light brushing, and a clean cloth was used for gentle drying. The floor was dry by the next morning and another replicate was set up the next day. Therefore, each trial lasted a full day, there was a second full day with no trial, and the next trial was conducted on the third day. Fresh traps with food baits were placed on the floor and rack shelves to capture any mites walking in the room on the non-test days that survived after the cleaning. Experiments were then continued on the next day based on random selection of a barrier treatment. I waited 24 hr after cleaning before another replicate was started. Therefore, there were five replicates in each of two experiments, and each experiment was deployed in completely randomized design over several weeks. A typical work week would have mite infestation of the treated rooms on Monday, Wednesday and Friday, with cleaning and drying on Tuesdays, Thursdays and Saturdays.

Traps for Sampling Mite Numbers

The mite traps were made from plastic Petri dishes that were modified by having mite entrance holes along the side wall and with a food bait inside and were the same design used by Amoah et al. (2017). The trap was made from a plastic Petri dish, 100 mm in diameter and 15

mm high. It was spray-painted black on the outside. Eight holes of approximately 0.5 mm in diameter were evenly-spaced at 2 mm from the bottom along the side of the dish to allow mites to enter contact the food bait. The bait was a 10 g circular disc, approximately 30 mm in diameter and 10 mm high, as described by Amoah et al. (2017) and similar to the rearing diet described in Chapter 2.

Trap Placement and Barrier Set-up in the Aging Room

For each trial a trap was placed vertically on one side of each of the hanging hams using a chain of rubber bands to form a belt across the ham. Each trap allowed a consistent way to sample floor-released mites that had climbed up the rack to a ham during the 24-h trial. The mites in each trap were counted in the laboratory under a dissecting microscope. Another sampling procedure was to make a 10-sec visual count of mites walking over a 7 cm section at two locations on each of the four rack legs one on the upper section and the other on the bottom half of the rack. A circular ring of 6cm diameter to put at a spot on the rack leg for 10 secs and mites in the circle were counted using a hand magnifying glass lens to see the mites. This was one on all the four legs of the rack and two spots for each leg. No traps were placed on the floor or racks in these experiments.

Approximately 10,000 laboratory reared mixed life stage mobile ham mites were released by scooping mites from active colonies using a tea spoon. To estimate mite numbers before the beginning of the simulated aging room experiments, mites were placed in a Petri dish, weighed on a scale then cooled to prevent them from moving to count them. After which the total number of mites were estimated under a dissecting microscope. This estimation of mite numbers was done before the main experiment was started. From then mites were released based on the weight of mites on the teaspoon for the experiments in the aging room without cooling the mites to keep

them active. Mites were obtained from a 3-week-old colony and released at the two ends of the room as shown in Fig. 4.1. The room was set up with cleaned hams, mites, baited traps and the experimental barrier treatment and checked the next day when the experiment was stopped at 24 h. All replicates were conducted under total darkness at 28 ± 2 °C and 65 ± 5 % relative humidity. Data loggers (Onset HOBO MX temp/RH logger, Bourne-MA, USA) were put in the rooms to ensure that temperature and RH stayed the same, while the experiment was ongoing.

Experimental liquid treatments were applied to the bottom legs of the ham rack by pouring about 2000 ml into plastic mixing bowls which was 27 cm wide diameter from the top part, 22 cm from the bottom and 9 cm high were obtained and the test was evaluated for its ability to prevent mites from walking up the legs of the rack and onto a hanging ham. The most effective and presumably lowest cost treatments were used for the simulated aging room studies which included soapy water, only water and vegetable oil as treatments and empty bowl with no liquid as control. There were five replications, each performed on a different day as described above, with the order of treatments randomized. At the end of a 24 h trial the traps were taken from the ham and the mites counted. The mites on surfaces of each ham were inspected using a hand lens (30 times HD LED magnifying glass; Model: L102; Company-AIXPI, Guangdong Province, China) during data collection and after cleaning to make sure there were no mites on the hams nor the rubber bands used.

An experiment on the efficacy of Tangle trap as a barrier vs no barrier were used in plastic bowls under rack legs. Alternatively, each leg was placed on top of a sheet of construction paper, 150 mm x 120 mm of length x width, that was fixed on the floor of the room with double sided tape. Tangle trap applications of 500 ml were placed on the upper surface of each sheet of paper and then brushed thinly over the complete exposed top surface. Each leg of the rack was

then placed at the center of each sheet of construction paper. The untreated control for this experiment had only the construction paper with noTangle trap coating.

Data Analysis

Data was recorded after 24 h for each barrier treatment and each experiment had five replicates in a balanced design. Mites in the traps from the aging room experiments and from food bait and plates the lab experiments, were counted and summarized across all treatments by calculating means and standard errors. Data were analyzed using the PROC GLIMMIX procedure in SAS version 9.4 (SAS Institute Inc. 2010). It was then submitted separately one-way and two-way ANOVAs using GLM models respectively. The types of barriers were compared to determine the effect of the barriers on the number of mites captures in the monitoring trap, or counted on rack legs, or in bait dishes for the lab studies. Treatment means were separated using Fisher's protected least significant difference (LSD) at the 0.05 significance level. SigmaPlot Version 12 was used to draw graphs for results as needed after the data were analyzed.

Results

Results for Laboratory Barrier Experiments

For the first experiment conducted in the plastic container, mite numbers that reached the food source were significantly higher when there was no treatment to serve as a barrier (108.0 ± 3.2 mites) than when there was a barrier (range from 0.0 ± 0.0 to 41.4 ± 3.2) (Fig 4.2). Vegetable oil was the most effective barrier at reducing mite populations, with zero mites on the diet. Soapy water was significantly effective than only water and Vaseline (petroleum jelly) treatments. However, the later was not statistically different from barriers created with mineral oil and soapy

water, although number-wise, mite numbers that crossed mineral oil to the diet was as low 1.8 ± 0.9 , soapy water was 11.2 ± 1.1 and Vaseline was 23.0 ± 6.3 .

In the second experiment, I used a taller plastic bowl that is 6cm high and treated with the various barriers, the number of mites that moved to the food source were statistically similar with the exception of only water and no water. They all had a significantly lower number of mites that crossed the barriers to the food sources. No water treatment had the highest mite numbers on the food source (ham). The control had a higher mean of 107.4 ± 3.6 mites on the diet which was distinctly higher than all the other treatments. Mean number of mites was reduced with water as barrier, although mites had to overcome a height, they did make it to the diet with a mean number of 31.2 ± 4.8 which was statistically higher than the rest of the treatments. However, all the other treatments reduced mites that moved to the food bait with a mean range of 12 ± 1.7 to 3.4 ± 0.7 with lower mean numbers recorded with the vegetable oil as a treatment.

In order to simulate legs of racks in the aging room, wood cut so that it was 6 cm high was used with mite bait resting on it (Fig 4.4). Glycerol, mineral oil and vegetable oil were the barriers that significantly reduced ham mite population from reaching the bait; followed by soapy water, petroleum jelly and then only water. Like all the other experiments, mite population that reached the bait were significantly higher in the no water treatment with mean number of 104.4 ± 2.4 because mite migrated without hindrance to the food source. Mite numbers ranged from 0.4 ± 0.2 – 40.6 ± 3.6 for all the other treatments.

With this experiment, tangle trap coating was used in the plastic container experiment instead of the liquid treatments. When I used a plastic bowl of height 7 cm to keep food bait away, mean mite numbers that made it to the food sources were 1.4 ± 1.0 whereas the control was 100.8 ± 2.6 . With the wood of height 7cm, tangle trap coatings still protected the food bait

and only 1.2 ± 0.3 mites made it to the food bait whereas the control with no tangle trap coatings contained 99.0 ± 1.6 mites. However, when Petri dish of no height was used with the tangle trap coating on the construction paper, double the amount of mean number of mites made it to the food source, 2.4 ± 1.1 but the control remained significantly greater at, 104.6 ± 5.6 comparably.

Results of Barrier Experiment in Simulated Room

Using a simulated room gives an idea of how best the aging room can be protected from ham mites and how to prevent them from crawling to the legs of the racks on the ham pieces. Regarding this experiment as always, empty bowls without any liquid treatments recorded the highest mean number of ham mites with 63.6 ± 1.8 for traps on ham and 42.6 ± 3.1 (Fig. 4.5) were mite samples on rack legs. However, mean number of ham mites reduced drastically for each liquid treatment. Mean number of mites on traps on ham that were recovered when liquid treatment was only water was 22.8 ± 1.9 which was significantly lower than the control where there was no treatment. Soapy water as 18.0 ± 1.2 was not significantly different from the only water neither vegetable oil treatments which was 11.0 ± 1.0 . However, all the treatments had a significantly lower mean number of mites when compared to the empty bowl, which was the control.

With regards to mean number of mites captured on the different spots of the rack, the same trend was found. Where there was no treatment, control with empty bowl there was a higher number of mites whereas all other treatments were significantly lower with 16.6 ± 1.5 , 6.4 ± 0.2 and 5.4 ± 0.7 (Fig. 4.6) being mean number of mites on racks for only water, soapy water and vegetable oil treatments.

With each treatment, the mean number of mites were less than the control that had no treatments or the empty bowl making the treatment very viable to prevent ham mites from reaching the ham pieces since even though not a total control, numbers reduced drastically. In Fig. 4.7, the tangle trap coating seemed to be the best way of keeping ham mites from getting to the ham pieces. Whereas the control is 47.0 ± 1.7 , traps with tangle trap coatings at the base of the rack reduced ham mite numbers to 2.8 ± 0.6 . The number of mites with no tangle trap coating was 21.4 ± 1.6 , and the treated base of the rack was significantly less at 1.4 ± 0.3 .

Discussion

Physical control is the use of physical tools that alter the environment thereby making it inaccessible or unfavorable for insect pests (Banks 1976, Boiteau and Vernon 2001). The present study indicates that physical barriers can be a tool to manage ham mites in an aging room. In all the studies, both the liquid treatments and the Tangle trap coating significantly reduced mites that migrated from the floor to the ham that was hung on the rack. The results suggest physical barriers should be considered to be included in the establishment of a viable pest exclusion scheme for dry cured ham in an aging room. It was evident in this study that the various treatments reduced the number of ham mites by obstructing their movement and most of mites died in these treatments.

Creating barriers using repellents and other food safe compounds on the ham pieces or incorporating into netting to protect ham have been proven to be effective to some extent in an aging room (Zhao et al. 2015, Abbar et al. 2016, and Manu et al. 2021). The combination of these two methods with effective monitoring as well as other tools developed over the years will contribute to the management of ham mites (Amoah et al. 2016).

Some of the physical obstacles used in the current experiments have been used in our laboratory protocols for rearing mites. The use of soapy water in a plastic container as a moat has been successful in our rearing process and also for various bioassays conducted with ham mites. Soapy water has less surface tension of the water is broken, mites that escape from the colony jars drown and are suspended on the sides of the container in the soapy water and are thereby unable to get outside. In this study, when soapy water was used in both the laboratory arena and simulated aging room, ham mite numbers that made it to the food source were less than the control where no soapy water was used as well as the treatment with only water without a detergent (Figures 4.2, 4.3, 4.4, and 4.6).

Some studies have shown that the soapy water either suffocates or breaks down the cuticle of soft bodied insect pests such as the aphids and house dust mites thereby killing them. However the mechanism involved in this process is not well understood (Smith 2021, Cranshaw 2022). Insecticidal detergents have been discovered to control insects on plants and have proven to be efficient in plant houses and fields (Borden and Dale 2019). Although dawn dish soap which was used in our experiments is not an insecticidal soap and while it was not a hundred percent deterrent, the number of mites that made it to the ham was dramatically reduced and could be used in combination with other tactics.

Another physical barrier that has been used both in plant protection and the packaging of processed food is the use of oils (Maarsin et al. 2020). Studies have shown that oils work by obstructing the spiracles, or breathing pores, along the sides of adult and larvae abdomens, and oil treatment quickly establishes a physical barrier to respiration in insects (Chalker-Scott 2008). Most of the oils used for the control method against mites are mostly essential oils from plants, which serve as repellents against the insect and/or mite pests (Wong et al. 2005, Mullen et al.

2012, Campbell et al. 2020, Manu et al. 2021). I employed vegetable oil as a barrier in our research and found that after 24 hours of treatment, the majority of the mites that migrated towards the food source were dead at the bottom of the container, and mite numbers were significantly reduced. When compared to other liquid treatments and the control, vegetable oil was the most effective at reducing mite numbers as a physical barrier when I did the laboratory arena test as well as the simulated infested ham aging room experiment (Figs 4.2, 4.3, 4.4 and 4.6).

Insects and mites both use movement to explore new food supplies, new regions for each individual, and new spots for females to lay eggs, resulting in an ever-expanding contaminated domain thereby when their movement is limited, they are unable to obtain these resources (Jian, 2018). Adhesives have been employed in pheromone-baited flight traps for moths and other insect pests that fly, where the insects fly and land in the traps, and the adhesives keep them there (Ferracini et al. 2020, Manu et al. 2018). The notion of a sticky trap (Green 2018) was applied in this sticky treatment, in which Tangle trap coating adhesive was used to prevent mites from reaching the hams. When compared to the control, the number of mites moving across the sticky coated surface (Fig 4.5 and 4.7) as well as mites on the rack decreased. According to Feat et al. 2019, adhesives and slippery surfaces can make it difficult for crawling insects to move. Some of the *T. putrescentiae* was that some of them walked onto the sticky coating surface and became stuck (Mullen and Oconnor 2002, Fayaz et al. 2016), whilst others fully avoided the sticky coating by moving away from it. It has been reported that body hairs, like those of the honey bee, create firm adhesion to surfaces (Fuller and Tabor 1975, Persson 2007, Endlein and Federle 2015) and that was seen with the ham mites too. In a study by Feat et al. (2019), it was discovered that depending on the type of adhesive coating used, applying coatings on surfaces

can limit the migration of insect pests. These coatings must, however, be long-lasting, simple to apply, low-cost, and environmentally benign (Ghosh 2006). Our sticky coating meets these requirements, and the fact that it was tested in a simulated aging room shows that this form of mite control might be able to be used in a commercial curing and aging room to help reduce mites crawling to aged hams. Although a physical barrier using adhesive coating may be a viable strategy for obstructing and reducing mite numbers due to their inability to crawl, not all insect or mite pests can be managed using this method due to attachment and friction forces, as well as species and pad type (Bullock 2009, Voigt et al. 2008). Hence, the type of coating and the substrate on which it is applied for successful control needs special attention. A disadvantage that was detected with our adhesive when I did preliminary tests was that when the temperature was extremely high above 35 °C the coatings melted and the effectiveness was reduced.

Physical control techniques have been used over time to manage stored product pests since the use of fumigants are slowly dying out due to health and environmental safety reasons (Kuhn-Archiv 2010). Some of the methods that are now being advocated for in storage facilities include, extreme heat treatment and cold treatment (Fields 1992, Vincent et al. 2003, Garcia 2004), package treatment and use of different types of packing which are insect-proof (Riudavets et al., 2007) as well as bioacoustics to detect presence of feeding larvae hidden in a grain structure (Welp and Reichmuth 1994, Hagstrum et al. 2012) which can also be used in the ham aging facilities. The use of liquid or sticky surfaces from this study can be included in these techniques as a control strategy for mites.

In as much as physical barriers have been successful in agricultural fields, they cannot be used alone to eradicate pest populations so there is a need for other tools that can be use

synonymously with barriers to have a successful pest management program (Boiteau 2002) and same applies to pest management in stored product facilities.

In conclusion, the results presented here suggests that using physical barriers such as soapy water, vegetable oil, and sticky coating surfaces could be considered as alternative techniques that are in an integrated pest management program. These treatments have minimal effects to human health non-target organisms and the environment. However, additional research can be conducted to determine the most suitable sticky coatings for ham mites and also different types of soap or even insecticidal soaps that can best prevent ham mites from reaching ham on racks in an aging room. For future work, these physical barriers could be used in ham aging plants to ascertain their effectiveness in a real-life ham aging room.

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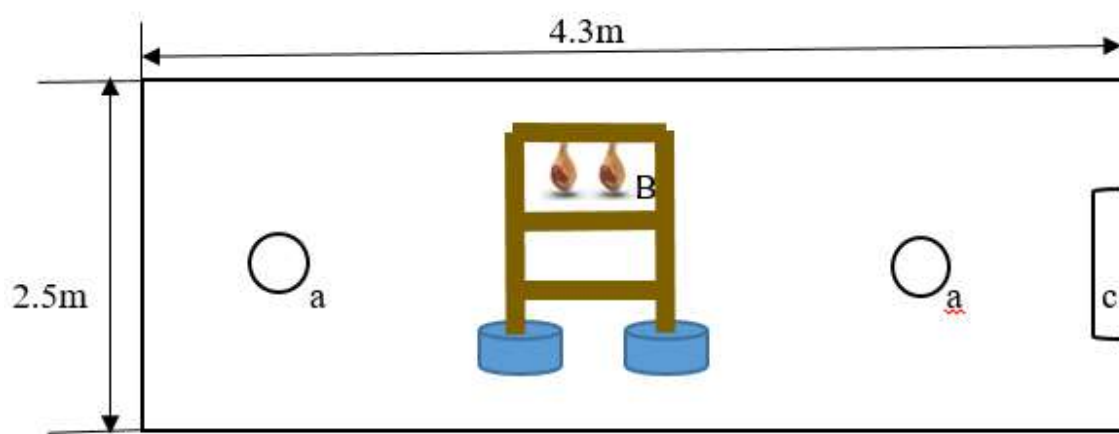


Figure 4.1. Diagram showing an illustration of the simulated dry cured ham aging room. a) Spot where mites are released B) Three-level metal rack with two hanging dry-cured hams and legs placed at the center of plastic bowl which contain the liquid barrier treatments c) Door to access the room.

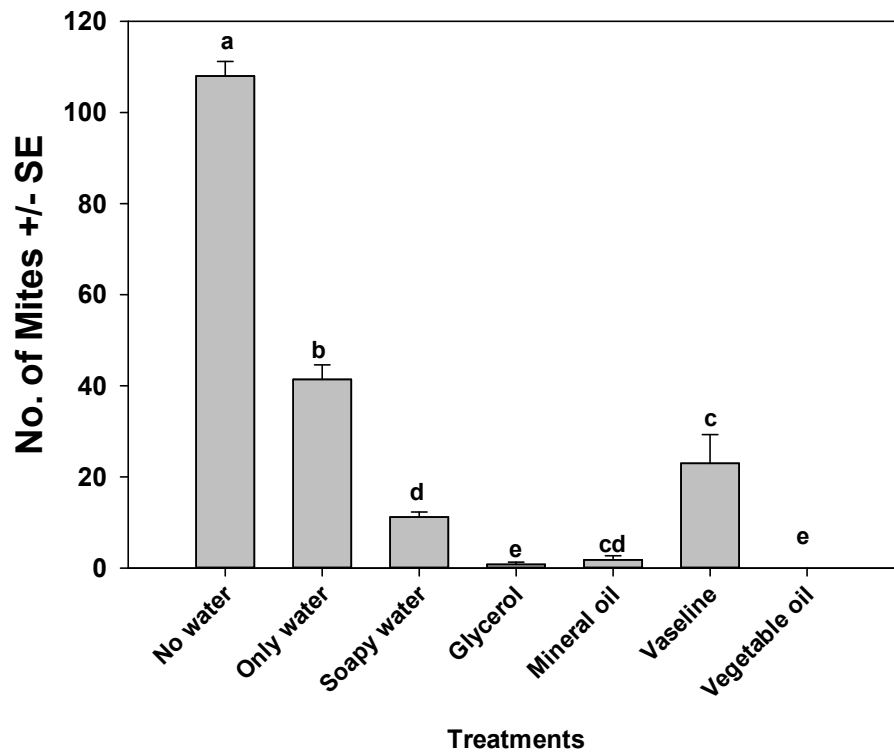


Figure 4.2 Graph showing ham mite numbers that overcame the various treatments of barriers to the food bait in laboratory plastic container arena after 24 h. ANOVA showed that there was significant variation among different liquid barrier treatments tested (F-value 362.13; p-value < 0.0001). Mean number of mite captures ($\pm$ SE; n = 5) for all barrier treatments. Means with the same letter are not significantly different (LSD; $p > 0.05$).

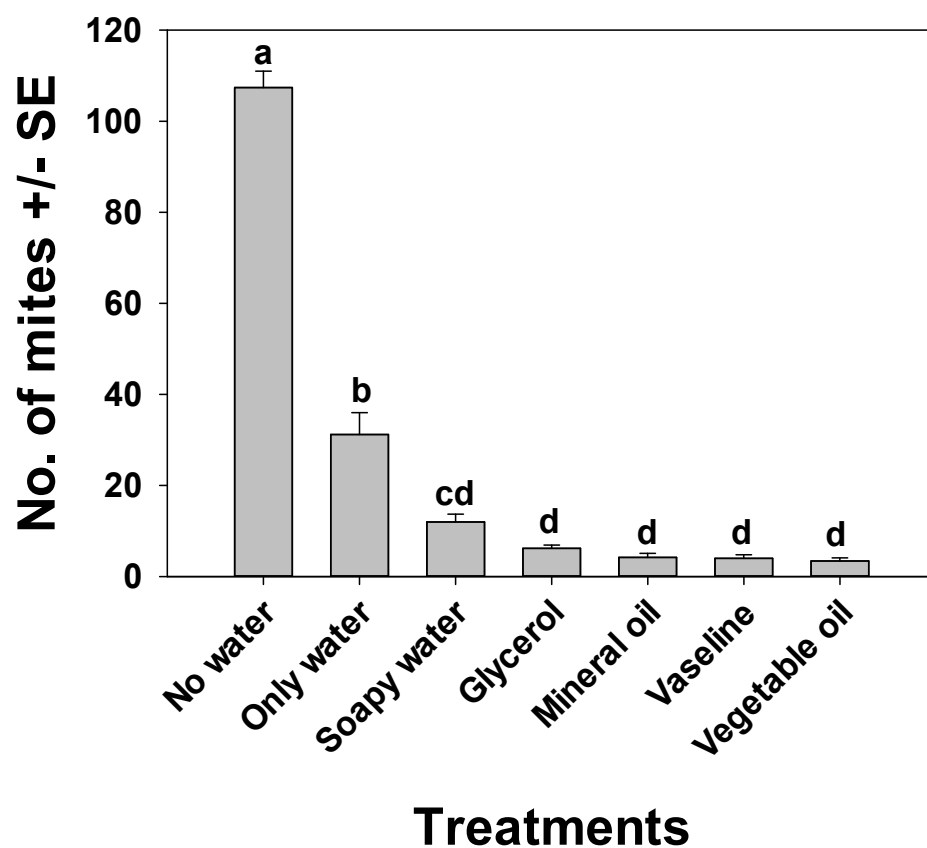


Figure 4.3. Ham mite numbers that overcame a height of 6cm plastic container across various treatments of barriers to the food bait in laboratory plastic container arena after 24 h. ANOVA showed that there was significant variation among different liquid barrier treatments tested (F-value 141.6; p-value < 0.0001). Mean number of mite captures ($\pm$ SE; n = 5) for all barrier treatments. Means with the same letter are not significantly different (LSD; $p > 0.05$).

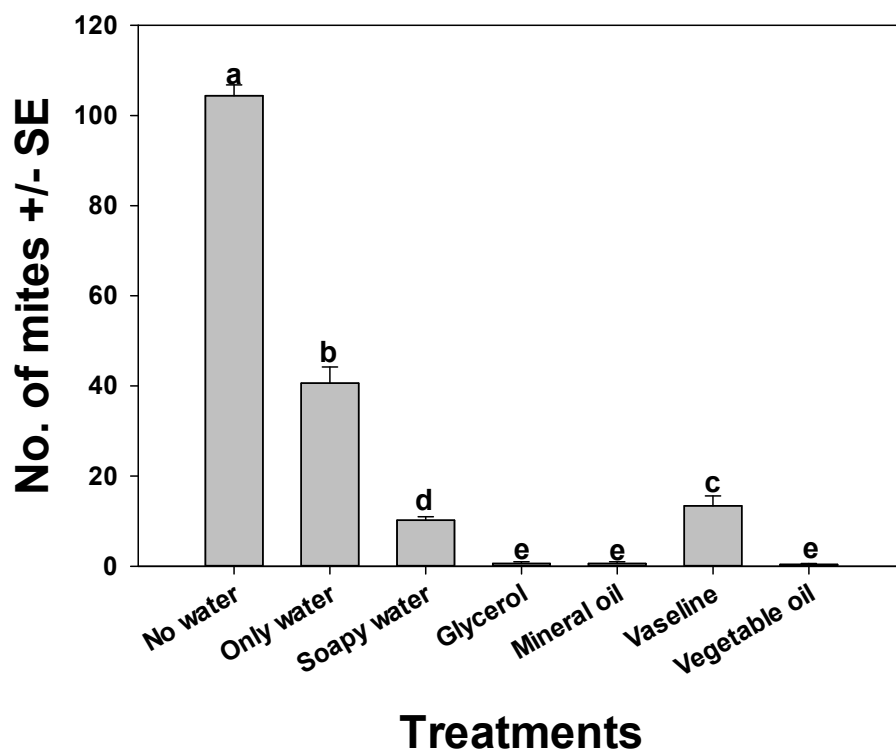


Figure 4.4. Ham mite numbers that overcame a height of 6cm and various treatments of barriers to the food bait which was suspended on a piece of wood in laboratory plastic container arena after 24 h. ANOVA showed that there was significant variation among different liquid barrier treatments tested (F-value 226.04; p-value < 0.0001). Mean mite Captures ($\pm$ SE; n = 5) for all barrier treatments. Means with the same letter are not significantly different (LSD; p > 0.05).

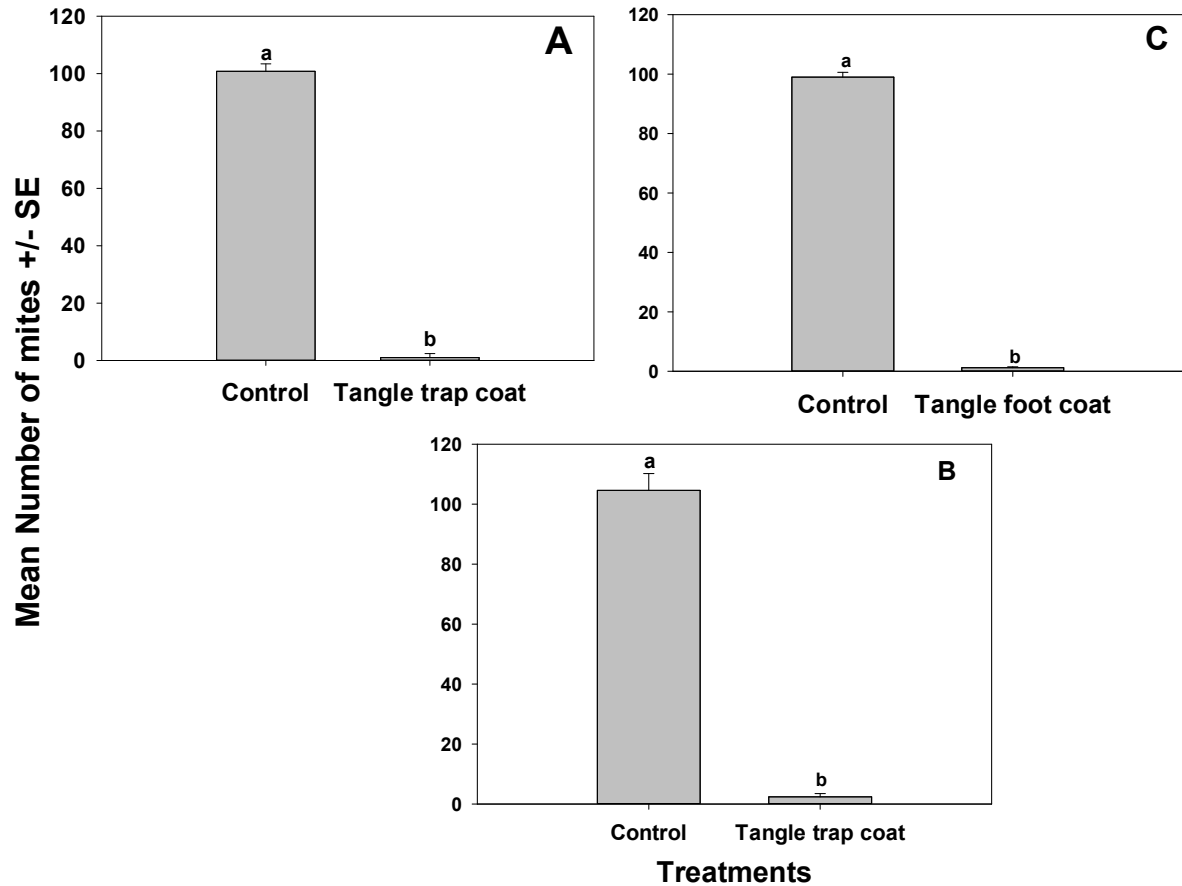


Figure 4.5. Number of mites that overcame physical barrier, Tangletrap coatings in laboratory plastic container arena after 24 hr. (A) Number of ham mites that made it to the food bait after 24 h after overcoming a piece of paper; 70 cm² coated with tangle foot coatings and petri dish of 20 cm² diameter with food bait at center (B) Number of ham mites that made it the food bait after 24 h after overcoming a piece of paper; 10 x 7 cm coated with tangle foot coatings with plastic bowl of height 7 cm containing a petri dish of 20 mm² diameter with food bait at center (C) Number of ham mites that made it the food bait after 24 h of overcoming a piece of paper; 10 x 7 cm coated with tangle trap coatings with a piece of wood of 7 cm high and food bait suspended on it. ANOVA showed that there was significant variation among dry barrier treatment and control (F-value range 1321.01–210.02; p-value < 0.0001). Mean number of mite captures (± SE; n = 5) for barrier treatments. Means with the same letter are not significantly different (LSD; p > 0.05).

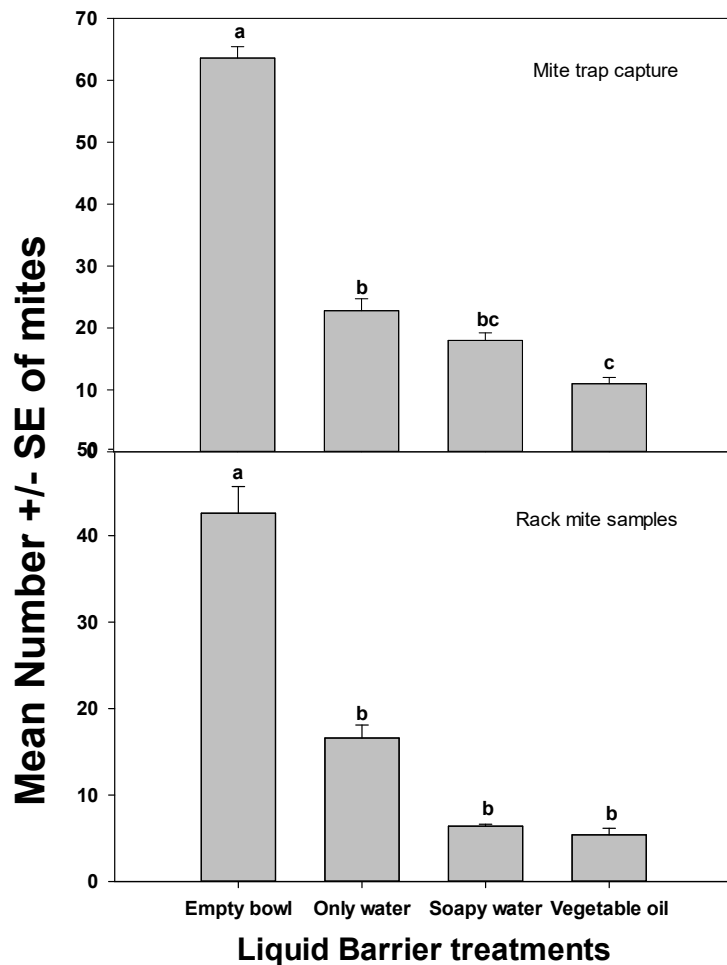


Figure 4.6 . The top graph shows number of mites that made it to traps on ham after 24 h using different liquid treatments (empty bowl, only water, soapy water and vegetable oil) at the base of the rack in a simulated aging room. The bottom graph shows mean number of mite samples that were randomly counted on different spots on the four legs of the rack in a simulated aging room. ANOVA showed that there was significant variation among different liquid barrier treatments tested (F-value 46.58; p-value < 0.0001) whereas for rack mite samples (F-value 19.35; p-value < 0.0001). Mean RIs ($\pm$ SE; n = 5) for all barrier treatments. Means with the same letter are not significantly different (LSD; p > 0.05).

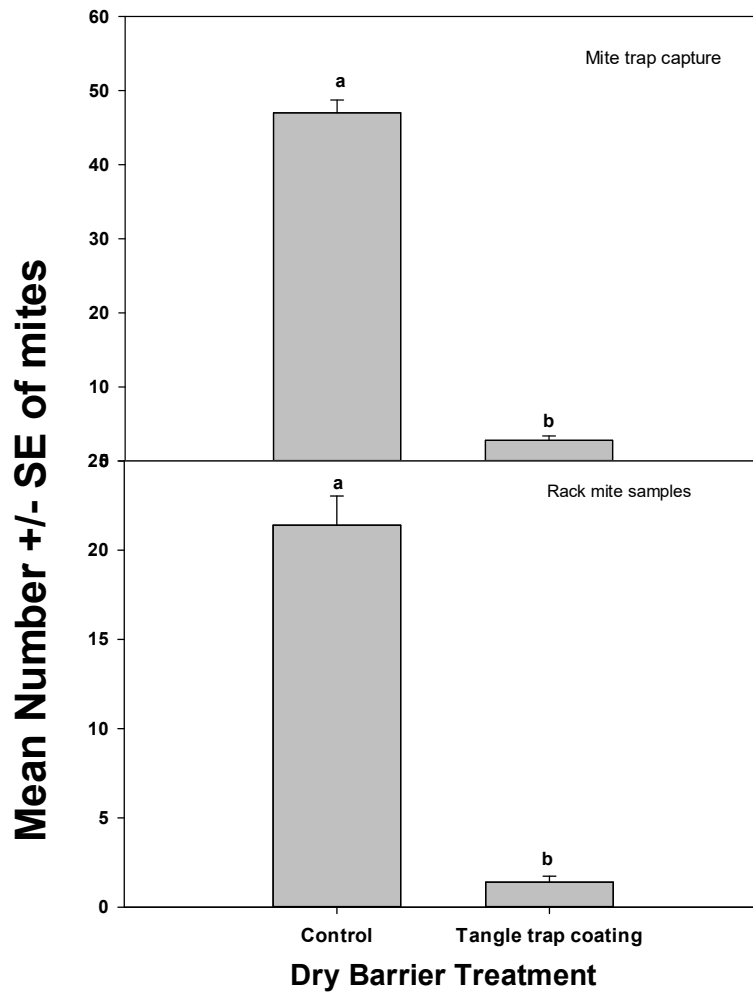


Figure 4.7. The top graph shows mean number of mites that made it to traps on ham after 24 h using tangle trap coating on construction paper as treatment at the base of the rack in a simulated aging room. The bottom graph shows mean number of mite samples that were randomly counted on different spots on the four legs of the rack. ANOVA showed that there was significant variation among dry barrier treatment and control tested (F-value 116.01; p-value < 0.0001) whereas for rack mite samples (F-value 28.74; p-value 0.0007). Mean RIs ($\pm$ SE; n = 5) for Tangletrap coating barrier treatments. Means with the same letter are not significantly different (LSD; $p > 0.05$).

Chapter 5 - Extension and Outreach: Mold mites, *Tyrophagus putrescentiae*, Pest of Durable Stored Food Products

Description

The mold mite, *Tyrophagus putrescentiae*, also referred to as the cheese mite or ham mites, has a very small body (0.5–3mm), eight walking legs, two body segments, although some lack body segmentation (Farrell 2018). Eggs are laid when females have an adequate and frequent food supply. Mold mites are very prolific. A single male mite can fertilize about 450 females, and a female can lay eggs within 24 h after mating as long as there is food and favorable environmental conditions (Gorham 1991). In a day, approximately 60 eggs can be laid by a female mold mite but 3-8 eggs/day are laid on average and about 70 % of these eggs are produced during the first three weeks of their life (Gorham 1991). Mold mites develop 11 days and can survive within weeks to a month. However, they can complete one generation in 10 to 24 days. The mold mite, *T. putrescentiae*, is fairly prevalent, yet normally remains unnoticed unless it becomes abundant. When initially seen, severe mold mite infestations can be mistaken for a pile of dust on the product until a closer look notices their movement.



Figure 5.1. An adult mold mite



Figure 5.2. Mixed life-stages of mold mites

Economic Importance of Mold Mites

The mold mite, *Tyrophagus putrescentiae* (Schränk) (Sarcoptiformes: Acaridae) is an economically important pest of stored products which are rich in protein and fat. Although, it is well known to be the primary pest of dry cured ham, it also infests other equally important high-value stored products such as other dried meats, cheeses, nuts, dried fruits, spices, grains and seeds, flour, cultured cheeses, straw, wallpaper, furniture, and semi-moist pet foods. Economic loss can sometimes occur from penetration of mites into the food (Rentfrow et al. 2012). *T. putrescentiae* can grow well in damp or humid environments. Dry foods with high moisture are prone to mold and provide an ideal nesting environment for storage mites. For instance, dry-cured ham and cheese aging chambers are prone to mold growth and arthropod infestations, especially mites, which can turn their surface to dust and lead to progressive deterioration (Principato et al. 2018).

Cheese

Infestations of mold mites have frequently been seen in cheese production during the ripening process, a phenomenon linked to exposure to contaminated environmental locations (Deong and Roadhouse 1922, Robertson 1952). Since *T. putrescentiae* are mycophagous and the fungus species digested by them are determined according to the digestive enzymatic properties of each species, it is documented that the symbiotic association between mites and fungi during the cheese ripening process may enhance mite's access to the cheese's rinds (Peace 1983, Marcellino and Benson 2013). This leads to economic losses and health problems in the cheese industry (Deong and Roadhouse 1922, Robertson 1952, Bell 2014). However, it has also been reported that the mites give flavor and other favorable characteristics to the cheese (Bruckner and Heethoff, 2016).

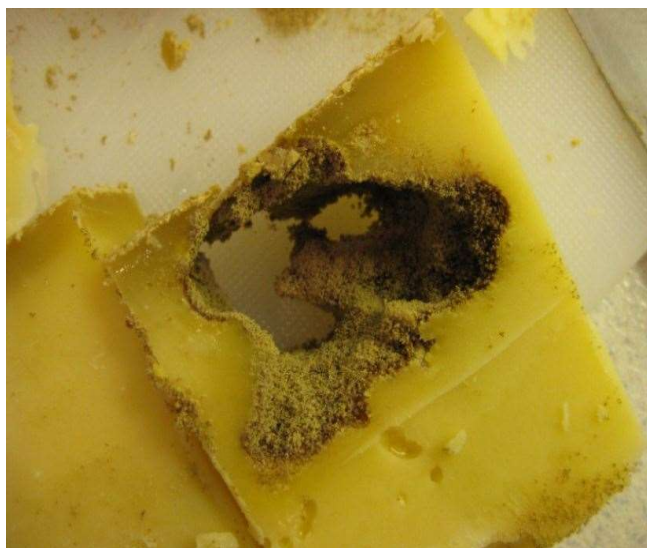


Figure 5.3. Mold mites' infestation on cheese (Image by <https://www.flickr.com/photos/34234018@N00/2622122438>)

Mite infestation in dried meat

Dried meat such as dry-cured ham may be infested by mold mites during the aging process, which is done to obtain the preferred flavor and taste. There is little to no infestation in ham that has been matured for three months or less (Rentfrow et al. 2006). More than six months of age should be taken into consideration in order to create the right flavor and product quality for a niche, high-priced market (Zhao et al. 2016). The likelihood of a mite infestation increases when dry-cured hams are aged for more than five months. However, a shorter lifespan of less than five months does not guarantee that there won't be any infestation problems (Rentfrow et al. 2006). In recent years, the amount of data and information available on storage mite allergies on human and animals has increased which is attributed to mite infestations. Mold mites infest dry cured hams during the aging process because the environmental conditions are between 16–25 °C temperature and 65–80 % relative humidity; is also favorable for mite growth (Toldra 2010).



Figure 5.4. Mold mites aggregated on dry cured ham

(Image by <http://extension.msstate.edu/publications/mite-control-dry-cured-ham-aging-facilities>)

Mite infestation in Semi-moist pet food

Intermediate moisture and low moisture food categories include semi-moist and low moisture pet foods, such as chewy dog treats and dog kibbles, respectively. A smaller but substantial component of the manufactured pet food market is made up of semi-moist pet diets, which calls for the use of humectants and acidity to regulate water activity and mold growth. However, mite infestation is prevalent in these industries during processing and storage. Pet owners purchase semi-moist pet feeds because they are highly attractive to animals and have minimal fiber and relatively high sugar contents (Zicker 2008). Growth of fungi on semi-moist pet food contribute to mold mite infestation which leads to food spoilage thereby causing economic losses.

Processed pet food contains food additives to improve nutrition, guarantee food safety, and impart desirable color, flavor, texture, stability, and spoiling resistance (Craig 2021). Many semi-moist dog diets and treats contain propylene glycol (PG), a man-made humectant and preservative (Aldridge 2014), which is very effective at controlling mold mite growth.



Figure 5.5. Mold mite infestation of a bag of semi-moist pet food (Image by <https://www.walesonline.co.uk/news/wales-news/grain-mites-infest-family-homes-17610636>)

PG is approved by the FDA for use as a general animal feed ingredient in most animal species with the exception of cats. It is designated as "Generally Recognized as Safe" (GRAS) (FDA 2012, 2018, FDA 2020). It was initially used to control mold mites in pet foods. However according to Christopher et al. (1989) and Hickman et al. (1990), PG can lead to hematological problems in cats which causes their death. Hence, the American Food and Drink Administration has outlawed the usage of PG in cat food in the USA (FDA 2020a).

Grains

Generally, mites are found in damp or moist grain, residues, oilseeds, and animal feeds however mites are a significant nuisance in some grain storage facilities. Mold mites directly harm grain products by consuming the grain germ and dispersing fungus in the commodities. When a heavily infested commodity is moved or processed, the off-flavors produced by squashed mite bodies contaminate it, which contributes to economic loss (Nayak 2019).



Figure 5.6. Mite infestation on soyabean (<https://www.familyhandyman.com/article/mold-mites/>
Image by Tomasz Klejdysz/Getty Images)

Mites have been an issue over the years, but they have been effectively managed by employing methyl bromide, which is a fast-acting and highly effective fumigant (Sekon et al. 2010).

However, due to its harmful effects on the ozone layer of the earth's atmosphere, methyl bromide is being phased out of use (EPA, 2006). The objective is to develop feasible and workable integrated pest management that are sustainable, environmentally friendly and safe to humans using the various baseline laboratory experimental tools developed over the years. *T.*

putrescentiae is one stored product pest that is difficult to control due to its small size and how prolific they are (Gorham, 1991). Any Integrated Pest Management (IPM) program that targets stored-product pests must include prevention, monitoring, decision-making, and management as fundamental elements. Identifying the pest species to be managed, understanding its biology and behavior, and determining the most appropriate and effective control approach or methods are all

important factors (Hagstrum and Subramanyam, 2006) to consider in an integrated pest management program.

This report covers recent baseline discoveries as well as currently available techniques for controlling mold mite infestations in dry-cured ham operations. Although the methods are laboratory tested for managing mold mites as a pest of dry cured hams, most of these methods have not yet been used in a commercialized facility. Further work in a commercial ham or these high value product processing facilities for some of these methods need to be conducted before they can be officially accepted for use in the industries if proven effective on a larger scale trial. The majority of these strategies can also be used to control mold mites of other stored products and these approaches can be interchangeable with those used to control other insect pests in stored products.

Prevention

Sanitation

Sanitation is a tool that is essential in any food processing facility. An important factor in avoiding mold mites in any food facility is sanitation. Lack of sanitation can cost a food processing facility money. Good sanitation practices and Sanitation Standard Operating Procedures, good manufacturing practices, and Hazardous Analysis and Critical Control Points plans must be established and followed. Sanitation as the foundation for a good storage program for any stored commodity. Thorough cleaning treatments include sweeping and cleaning floor areas with hot water and alkaline cleaner, vacuuming and scrubbing surfaces with soapy water, replacing broken racks, sanitizing the room with quaternary ammonium compounds, sealing cracks and crevices to prevent pests from hiding and limiting employee access to the facility (Schuler et al. 1999, Campbell et al. 2021).

Racks, walls, floors, ceilings, and equipment need to be thoroughly cleaned in the facility. The same attention being given to the empty rooms needs to be given to the equipment in the room for product storage in order to prevent any harborages for the mites. Cleaning should be done at least twice a year, (spring and fall). When cleaning and sanitizing is finished and the room is dry, the products can then be brought into the room.

If possible, replace wooden racks with metal racks. For example, in a ham aging room, wooden racks tend to harbor grease and ham residues whereas metal racks are easier to clean. In situations where wooden racks are used, make sure there are no cracks since mites are very tiny and can hide in the wooden cracks. In grain storage facilities, the pallets harbor spillage of grains which can also build up mold mites. Hence removal of wooden pallets to clean them is important.

Personnel in these food industries are trained to maintain food and personal hygiene. It is important to enforce foot baths, hairnets, caps, and lab coats at each entrance to the storage room, and restrict employee and personnel access to the facility. Before cleaning, lab coats can be frozen for 24 h to eradicate any pests that might be on them.

In the cheese industries, floors and walls are constructed with durable and easy to clean materials such as concrete, dairy brick or tiles and the floor corners are curved to prevent bacterial and mite dust accumulation. Floors are also sloped to allow for easy water drainage and easy cleaning of the structure. Equipment and utensils for making cheese are made of durable easy to clean materials such as stainless steel, food safe plastic or rubber to prevent mites' harborages (Kaylegian, 2020).

Physical Barriers

This preventive method needs to be tested in the ham aging facilities before inclusion in the preventive measures. A simple experiment conducted in our laboratory indicated when the legs of the various racks on which ham is hung are placed in a liquid treatments or cardboard with sticky adhesive surfaces, mite numbers that climb to the shelves of the rack reduced drastically. Hence this could be added to the prevention methods after sanitation if they are effective in a commercial facility. The liquid barrier treatments that were used for our laboratory experiments included soapy water and vegetable oil as well as sticky adhesive coatings on cardboards were used. This could be the main methods for keeping ham mites away from the little pieces of meat, trimmings, and oil that may be left on the floors and shelves of an aging room. If this method is used, the barriers must be changed over time to prevent hoarding of mites in the treatments.

In food processing and storage facilities, rodents and other insects are major pests that can carry and spread mites. All vegetation and trash bins including weeds, turf grasses, trees, shrubs, and other objects must be kept away from the external walls of the buildings to prevent the spread of mites. It is important to make sure there are no spills or left over food pieces around the building to encourage other pest to visit the building. To remove a harbor zone for mites, rodents, and insects, a 1- to 2-foot "dead zone" made of gravel or a solid surface is advised. Sealing the opening found on the building's exterior after inspecting it is crucial to prevent infestation.

Light Treatment

Ham mites tend to stay away from light, especially red or white lights, fluorescent, or LED lighting, and instead prefer to be in darkness. This was evident in a laboratory trial conducted in

our lab. However, a large-scale test of these lights has not been conducted in a commercial food facility. If these lights are proven effective, use red LED lighting in aging rooms, buildings, and at entrances and doorways prevent mites' activity and all other facilities. When workers are in the rooms in the facilities it will be necessary for them to use white lighting to prevent mite activity. This method alone cannot totally control mites in a facility thus the need to use it in collaboration with other IPM methods.

Heat and Cold Treatments

The use of extreme temperatures has been used in some insect infested facilities but not in mite infested commercial facility. However, laboratory experiments have shown extreme temperatures such as cold and hot treatments can be used to control mold mites. Mold mites cannot endure extreme temperatures. By using heat as a remedy to remove mites after removing all the products from the storage facility can be incorporated into an Integrated Pest Management (IPM) program. Laboratory tests have indicated that to achieve this; 40 °C for 4 days, 42 °C for 2 days, or 56 °C for 1 day will reduce mites' numbers significantly (Abbar et al., 2016b). Before bringing in mite-free food products, thoroughly clean and sterilize the space after the heat treatment by using the sanitation methods described above. On the other hand, mold mites cannot survive in frigid temperature conditions. When using freezing units to chill a space of mite infestation, set the temperature to -15 °C for 12 hours, -10 °C for 1 day, -7 °C for 40 hours, or -5 °C for 2 days to kill ham mites as described by Abbar et al. (2016b). This can be very expensive so this method can be used by moving infected products to a walk-in freezer set at - 9 °C for 24 hours if you are unable to reduce the temperature of the entire facility to control mites.

Monitoring

There are commercial traps that can be used to estimate the amount of the mite population and determine when it is time to take action or to assess the impact of fumigation and other control measures on mite populations (Wakefield and Dunn 2005, Amoah et al. 2016). To evaluate several dry cured ham facilities in the Southeastern United States, Amoah et al. 2016 used food baited mite trap, a prototype of the "BT Trap" made in England (Thind 2005). Results in a commercialized aging room showed number of ham mites ranged from zero to several hundred per week over the course of two years. This trap is very easy to make, accessible and cheaper than the BT trap. However, it is uncertain if mites stay and remain in the trap or move in and out of trap. It is important to plan to take corrective action if trap captures indicate high mite population and observation samples surpass a threshold limit (Amoah et al. 2017). By using traps, it is easier to identify thresholds and the necessary actions be taken (Amoah et al. 2017).



Figure 5.7. A food-baited trap with the lid held on by a rubber band (Left) and moist food bait inside trap with mites feeding with tiny holes around the side to allow mites to enter (Right). (Image by <http://extension.msstate.edu/publications/mite-control-dry-cured-ham-aging-facilities>).

Mitigation

Chemical Control

The red-legged ham beetle, cheese skippers, and larder beetles are insect pests that may be present when mold mites infest dry meat, cheeses and semi-moist pet food facilities or when

mold mites' infestation are not present. With good hygiene, generally available pesticides, and fumigants, these pests can be efficiently controlled.

A few insecticides and acaricides are effective at sanitizing the area where these products are found. In a ham aging room there are metal, wood or concrete surfaces. Therefore, prototypes of these surfaces were created and tested in the laboratory since mites move by crawling on these surfaces. Abbar et al. (2016) reported that chlorphenapyr that killed 100 % of mites on all surfaces whereas a recent study which evaluated acaricides demonstrated that amitraz and acequinocyl were very effective at killing almost all ham mites on these surfaces with a range of 60-80 % mortality after 6 weeks of treatment. These chemicals lasted on the surfaces for about 6 weeks. If amitraz is for instance added to the management program, it may be necessary to reapply after every four to six weeks to keep their potency.

However, these chemicals are not registered for use around food hence there must be an approval for the FDA for use around food before it can be recommended for use in these facilities. Amitraz and acequinocyl are not currently registered to be used in storage facilities, more research into their safety around food products could warrant their use in these facilities. Additionally, they can be used in on rotation to chlorphenapyr which stayed on surfaces for more than six weeks and were still effective at killing mold mites to prevent mites from developing resistance to them. Therefore, these chemicals could be incorporated into the integrated pest management program for *T. putrescentiae* to control their population if approved for use but before then although I recommend, it must be approved for use.

With respect to chemicals for surface protection against mites in grain facilities, methoprene (eg. Riacon S[®], IGR[®]) is currently registered as a stored grain treatment for insects but from my recent research these growth regulators do not kill adult mold mites. Other chemicals that have

also been used in a grain facility include some pyrethrum and spinosad insecticide products, which can only last at most for two weeks on the surfaces to kill insects and mites (Nayak, 2019; Manu et al, yet to be published)

Natural and synthetic repellents and Food Safe Compounds

Preliminary laboratory tests indicated the growth of the mite population can be effectively slowed down by a number of food-grade coatings. These coatings include propylene glycol, lard, ethoxyquin, BHT, BHA, carrageenan, propylene glycol alginate, and xanthan gum combined with propylene glycol (Abbar et al., 2016a; Campbell et al., 2018; Campbell et al., 2020). Research suggests food-grade coatings incorporated in nets could protect the ham from mites. For now, incorporation of PG in nets is being commercialized however the other food safe compounds mentioned here are not tested on commercial basis. An Integrated Pest Management (IPM) strategy that focuses on mite prevention and control might include these nets. Another management tool that can be used in protecting products from mites is incorporating repellents.

Both natural and synthetic repellents and other food safe compounds can be incorporate into packages in which products are kept in order to repel mites that come close to the packages. An experiment conducted by Manu et al. (2021) in the laboratory indicated that some natural repellents such as citronella and carvacrol and C8910 which is a synthetic food-safe compound are effective at repelling mites. If a commercial test proves that these compounds repel mites on a larger scale and do not have any sensory impact on the food then this could be incorporated into the IPM program.

Fumigation

After the ban of methyl bromide, other fumigants have been researched for use in flour mills, processing facilities and retail shops to control stored product pests including mold mites.

Phosphine and sulphuryl fluoride were found to be very effective at killing mites. However they both had some limitations after application in a commercialized food processing facilities.

Phosphine fumigant was promising and approved for fumigating stored products including dry-cured ham facilities. The most significant benefits of phosphine are its ease of use, low cost, and potency. For stored products, the maximum permitted phosphine residue concentration is 0.01 ppm (USDA 1999). Phosphine fumigation at 2,000 parts per million at 24 °F or warmer is effective at killing all life stages of mold mites in 48 hours or less (Hasan et al., 2020). Whereas phosphine is very effective and could kill all life stages of mites, they corroded and damaged metal and copper items such as electrical appliances. Hence if it is considered to be used in a facility then it is advisable to seal these appliances tightly to prevent corrosion (Zhao et al., 2012).

Sulphuryl fluoride (ProFume) on the other hand could control mobile life stages of mites and not the eggs even after concentration were increased three times the recommended rate, the eggs still survive (Phillips and Schilling, 2013). Fumigants ethyl formate and propylene oxide have also been found to kill mites; however they are not registered for use yet in the USA hence cannot be added to the control calendar for mite control.

Ozone and Carbon Dioxide are also synthetic gases that can be employed for mite control. Ozone killed all life stages of mites at higher concentration of 175 and it is permitted for use in the United States arounds storage facilities (Guzel-Seydim et al. 2004; Hansan et al. 2016). Likewise, all life stages of mold mites died after 144 hours of exposure to 60 percent CO₂ (Hasan et al. 2016). Hence these two synthetic gases can be potentially used in the mold mite control programs.

In conclusion, although all these methods mentioned above are at the laboratory testing stage, they have great potential for use in the future either alone or in combination to solve the problem to mold mites' infestations in these food facilities. To establish a management program appropriate for stored products using these methods to successfully control the mold mites, *Tyrophagus putrescentiae*, most of these tactics would need further studies to confirm their efficacy although they are suggested here. It might not work as I saw in the laboratory experiments when I conduct similar studies in a commercialized area since laboratory conditions can be controlled but not so for an actual commercial facility. Other mold related pests of food in storage also need to be controlled to successfully achieve a complete uninfested facilities. In some situations, these IPM techniques should be utilized in conjunction with others for an effective approach to completely eradicate mold mites in any stored product facility.

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Summary of Proposed Integrated Pest Management Methods for Mold Mites, *Tyrophagus putrescentiae*

Below is the summary of all the methods that may be needed in a complete integrated pest strategies against mold mites as well explained above.

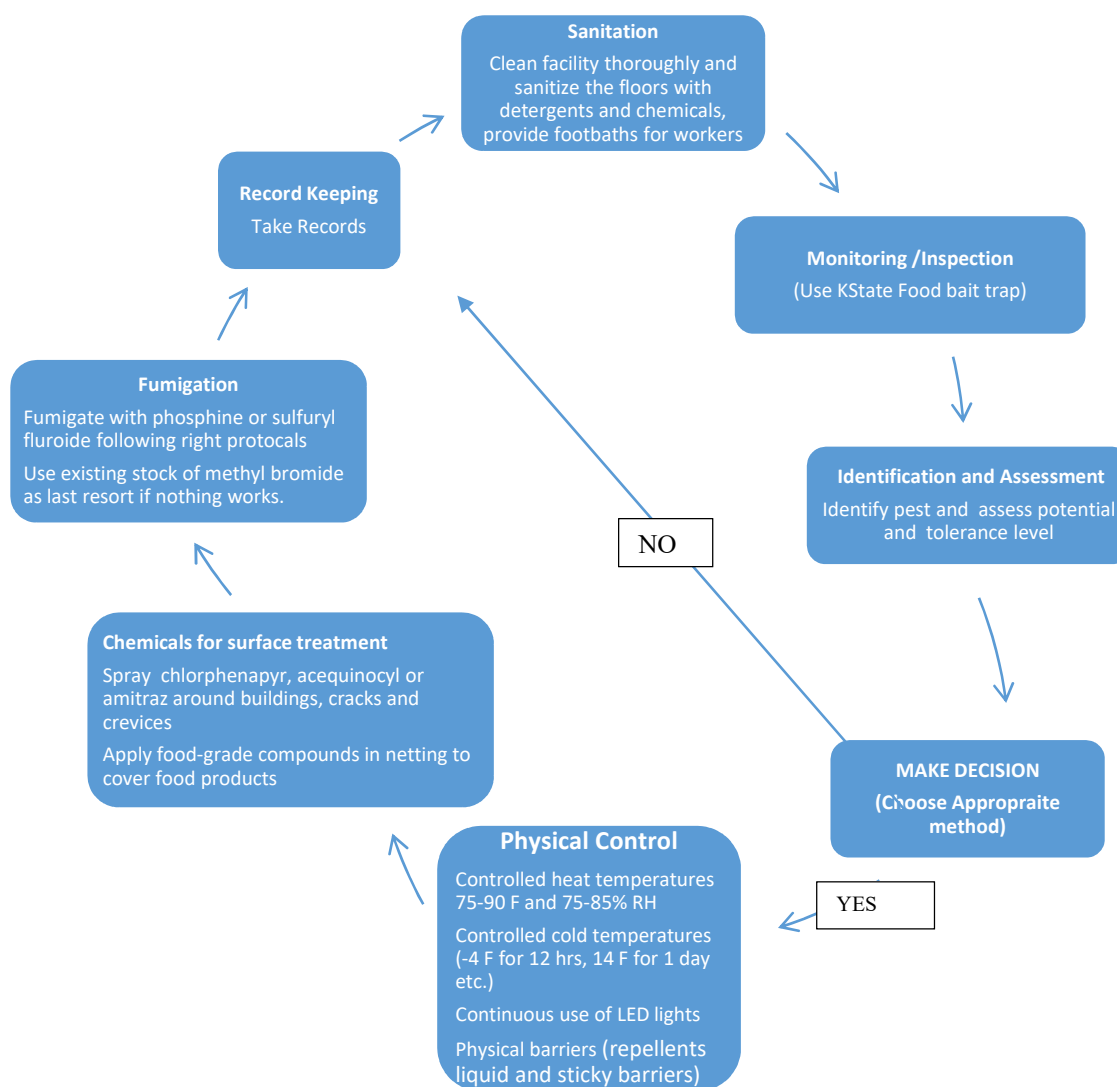


Figure 5.8. A cycle chart summarizing and highlighting major suggested IPM methods mentioned above in controlling mold mites.

Chapter 6 - Conclusions

In recent years, the research in seeking for integrated pest management tools for controlling mold mites has significantly increased, although the knowledge of using different methods aside fumigation for preserving ham has been in existence for a long period of time. The use of methyl bromide was the primary means to control mites in an aging room when processing dry cured ham however it is phased out since it has ozone depleting properties which cause the earth's atmospheric stratosphere to deplete. Southern dry cured ham represents a high – value specialty meat which is subjected to mites' infestation, there is the need to look for alternatives that will serve as a long term solution to this problem. Developing an integrated pest management (IPM) tools that is feasible, economically and environmentally friendly is the sure way to control this pest.

My dissertation focused on contributing to the knowledge of developing alternative tools that can come together to control mite infestations on dry-cured ham and other equally important storage products. Our study used food-safe compounds as repellents and also to reduce the population growth of mites on pieces on ham in chapter 2. In addition, different acaricides and insecticides were screened and evaluated for their residual efficacy on different surfaces in chapter 3. I also assessed different liquid and sticky adhesive surface coatings to serve as barriers against ham mites in chapter 4. Chapter 5 of the dissertation put together all the baseline methods as a proposed extension material for producers and the pest control industry to implement in dried ham IPM program in the future.

The overall findings of chapter 2 demonstrated some natural and synthetic essential oils can serve as repellents against mites and also significantly reduce mite population growth when pieces of ham were coated in these oils. I propose to further explore the use of these essential oils

by incorporating them into nets to protect dry-cured hams in a commercialized ham aging room which would contribute immensely to the knowledge on development of the tools against ham mites.

As the literature review in chapter 2 revealed food-safe compounds have been used in controlling stored product insect pests but not much has been done using these compounds against storage mites. The natural oils used included carvacrol, citronella, nootkatone and geraniol whereas the synthetic compounds were DEET (N, N-Diethyl-3-toluamide), Icaridin and C8910. Carvacrol, geraniol, and citronella were found to repel *Anopheles gambiae* (Diptera) successfully at lower concentrations (Omolo et al. 2004, Odalo et al. 2005). It was also discovered that the commercial synthetic insect repellent DEET is not only a broad-spectrum repellent and very important against mosquitoes (Isman 2006) however it had a very low repellency index against mold mites. The commercial formulation of C8910 is repellent against biting and non-biting flies such as mosquitoes and acarids such as ticks (Mullen et al., 2009) and it was the most effective at repelling mold mites for the synthetic oils. All these compounds in one way or other has been successfully used against insects and other acarids.

In this study, I explored the use of these compounds to manage ham mites. I demonstrated how these compounds repel mites on glass surfaces, their orientation towards or away from treated ham pieces and oviposition after 4 hrs. on these pieces in a choice assay. I also assessed how these compounds reduce mite population growth after twenty mites were forced to feed on treated ham pieces and the number of mites in jars were counted after 14 days.

The results of the choice assay showed that natural essential oils readily repel mites on the glass surfaces when they were allowed to move in a Petri dish. With regards to

orientation to treated ham, mites moved towards the nontreated ham pieces. I discovered the initial choice determined where mites will be and once, they made their choice, they tend to stay on the ham pieces throughout the different times I recorded their numbers. Another discovery was that, the repellency of mites by these compounds were concentration-dependent. As I increased the concentration of the compounds, mite numbers that moved away increased significantly. Geraniol was the most effective compound in repelling mites. A concentration of 0.04 mg/cm^3 resulted in a repellency index of 96 % for the natural oils whereas C8910 was the best at repelling mites compared to the other synthetic compounds however higher concentration of 0.1 mg/cm^3 was required. With regards to the population growth, mite numbers reduced drastically even below the initial 20 mites inoculated when 10 % of the compounds in solution was tested.

This study showed that the use of food-safe compounds such as terpenes from plant essential oils and a mixture of synthetic short-chain fatty acids might provide a reasonable way to effectively prevent the infestation of ham mites on dry-cured hams during aging. Food-safe compounds can be incorporated into integrated pest management programs as a preventive measure to control ham mite infestations, since methyl bromide fumigation is being phased out of use. Our study confirms the potential of these compounds to serve as repellents against ham mites on dry-cured ham.

Although mites are acarids, not much work has been done on evaluating acaricides against this pest. Hence our studies screened twelve EPA approved acaricides and insecticides known to control other mite pests in the horticultural and veterinary departments. The efficacy of some selected acaricides and insecticides were evaluated by screening and further studies to evaluate residual effects on wood, concrete and metal

surfaces were conducted. Chapter 3 described the screening of these chemicals in glass vials against mites and after the experiment, the most effective ones which in our case four chemicals including amitraz, acequinocyl, cyfluthrin, and pyrethrin + piperonylbutoxide were the most effective, with greater than 90 % mortality during the screening test against mold mites. This led to the selection of these chemicals for evaluation in subsequent studies to determine their effectiveness against mites on the different surfaces.

Our findings indicated that amitraz and acequinocyl which are used in horticulture, agriculture, and animal husbandry to control ectoparasites such as mites, fleas, lice, ticks etc. (EPA 2002, Gutpa 2007) were the most promising chemicals and lasted on all surfaces even after 6 weeks after application. If this chemical is being used in the future on surfaces, then there must be the need to reapply it after every 4–6 weeks. Currently, amitraz is registered to control *Varroa* mites in honeybee hives (EPA 2022). The development of this chemical and its inclusion as a tool for IPM of storage mites, including *T. putrescentiae*, is therefore possible. However, after the label is expanded to include this application on food and human safety issues with this compound are discussed. There is the need for more research to be done to determine how safe amitraz is when it is used around stored food products for mite control through residue testing in foods and on food contact surfaces.

In chapter 4, I evaluated liquid treatments as barriers and a sticky coating on surfaces to obstruct mite movement from hams on the rack. Our results suggest that, by using liquid treatments such as soapy water and vegetable oil, the number of mites that made it to the top of the rack to infest ham reduced significantly. Various laboratory studies to find liquids that are very promising and economically benign to control mites were done. After the laboratory experiments, I found mineral oil, vegetable oil and soapy water to be the best in controlling mites

that went to the food source. After I found the best liquid treatments, I used the treatments that were less expensive for further studies in simulated room.

Rack legs were placed in the center of a bowl with the various liquids as different treatments and no liquid in the bowl as a control in a simulated room. I found that by using these liquids, mites drowned or suffocated to death leading to significantly low numbers in the traps on the ham that were on the racks.

Another treatment, which I used Tangletrap® sticky coatings on surfaces of cardboard as treatment and nothing on the cardboard as control barrier were the most effective. However, when using adhesive coating, it is important to consider the type of adhesives and the stickiness. According to a study by Feat et al. (2019), adding coatings to surfaces can stop insect pests from migrating, depending on the type of adhesive coating employed. However, these coatings must be durable, easy to use, inexpensive, and eco-friendly (Ghosh 2006).

Based on our findings, I can conclude that using physical barriers, such as soapy water, vegetable oil, and sticky coated surfaces, could be taken into account as alternative strategies in an integrated pest management program. However, these are baseline experiments hence using these methods in a commercialized aging room would confirm our findings.

A draft of an Integrated Pest Management (IPM) program summarizing the various baseline tools that have been studied so far into an extension piece to benefit producers. This extension fact sheet will does not only cover how to control mites in a ham aging room but also for other economically important stored products like semi-moist pet food, grains, and chesses as describes in chapter 5.

The fact sheet covers tools that protect products through prevention, monitoring, decision-making, and management to control mites. Storage mites are difficult to control,

however with this guide, it will be easier for managers of processing and storage facilities to follow the program and make decision befitting for their situation in these facilities to control mites.

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