The effect of relative humidity on acaricide efficacy against and dispersal characteristics of the mold mite *Tyrophagus putrescentiae*

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Abstract

This study determined the effects of relative humidity on mold mites (*Tyrophagus putrescentiae*) with regard to acaricide efficacy and dispersal characteristics. The mold mite is a stored product pest that can be a problem in the retail habitat. Mold mite survival is dependent on relative humidity (RH), so different RH levels were used to determine the efficacy of several commercially available residual acaricides compatible with use in retail operations. To simulate retail shelving, acaricides were applied to Petri dishes and allowed to dry. Mites were placed in dishes, dishes were assigned to one of three RH levels, and mortality was recorded after 24 and 48 hours. Increasing humidity levels generally decreased acaricide efficacy. Resulting efficacy indicated that some residual acaricides may act as an effective barrier against this mite. A second study analyzed the dispersal characteristics of mold mites. Infestations may remain undetected in food sources for long periods, where populations may increase and food sources become depleted, resulting in dispersal of mites to new habitats that have suitable nutrition and humidity. Arenas were designed with an enclosed food reservoir filled with ground dog food and incubated at high humidity. The rest of the arena provided a space for mites leaving the reservoir, and this space was incubated at one of three RH levels. The number of mites leaving the food reservoir was monitored daily until dispersal mode and final population characteristics were determined. In this experiment, mites exhibited explosive dispersal, where migration occurred *en masse* within 24 h and was dependent on increasing mite density. These findings suggest that direct sampling of habitat may be necessary to detect infestations before explosive dispersal has occurred.

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Introduction

The mold mite, *Tyrophagus putrescentiae* (Schrank 1781) (Acari: Acaridae), is a stored product pest of economic significance that has been a problem in many types of food. This mite damages grains (Hughes 1976), copra (Hughes 1976), cheese in cheese houses (Robertson 1952), cured ham (Arnau & Guerrero 1994), buckwheat (Chmielewski 1999), mushrooms (Kheradmand et al. 2007), fungal cultures (Duek et al. 2001), and pet food (Brazis et al. 2008).

In the grocery retail industry, estimated losses attributed to mold mite infestations can be as high as $2000 per store. Assuming 5% of grocery stores within a chain can be affected, industry-wide damage amounts are estimated at $3.6 million (estimates calculated using data from Manta.com). Pet food stores are also affected by mold mite infestations, and, due to the higher volume of dog food in these stores, mold mite infestations may cost more than $2000 to remediate. This can result in direct costs of $20-$40 million annually. These estimates do not include potential long-term damage to brand identity. Additionally, the non-specificity of the mold mite's diet enables an infestation to spread to other commodities, further complicating control measures. Infestations by mites can occur at any point along the food manufacturing, storage, and distribution process, which increases the likelihood of exposure to humans and animals.

Under favorable conditions, the mite can accumulate into a large amount of biomass, far greater than most other pest species (CSIRO 2001). This rapid accumulation of mites leads to a substantial depletion of the commodity, causing significant economic losses (Žďárková 1991, as cited in Sánchez-Ramos et al. 2007). Mold mite infestations

cause substantial damage due to loss of commodity and cost of controlling infestations. In 2009, infestation of grain by various mite species resulted in the rejection of 1.52 million tones of malting barley (0.2% of the commodity) in the United Kingdom (Anon 2010, as cited in Collins 2012).

In addition to destroying food, there is evidence that this mite may be a source of allergens affecting dogs and humans (Mueller et al. 2005). Feces and exuviae of *T. putrescentiae* have been shown to contain 20 antigens and five allergens (Arlian et al. 1984, abstract). Exposure and allergic sensitivity to this mite may occur through respiration, epicutaneous contact, or ingestion (Gill et al. 2010). Symptoms and conditions in humans resulting from sensitization to *T. putrescentiae* include asthma, rhinitis, conjunctivitis, dermatitis, and, in extreme cases, anaphylaxis (Liao et al. 2010, Van Hage-Hamsten and Johansson 1992, Canfield and Wrenn 2009, Mueller et al. 2005). The allergic reaction is caused by an IgE-mediated immunological response (Van Hage-Hamsten and Johannson 1992). Along with house dust mites and other storage mites, *T. putrescentiae* has also been found in house dust samples, indicating that the exposure to allergens may not solely be occupational exposure associated with workers in grain storage and grocery stores (Gill et al. 2010, Van Hage-Hamsten and Johannson 1992). While it is believed that dogs with atopic dermatitis are especially prone to sensitivity from mites, intradermal testing of dogs in Colorado showed no significant difference in reactivity between dogs with and without atopic dermatitis (Mueller et al. 2005). However, these results conflict with other studies that found that dogs with atopic

dermatitis showed higher hypersensitivity to *T. putrescentiae* after intradermal testing (Hillier 2001).

This cosmopolitan mite is found in many environments, urban and rural, and can be free-living or parasitic. For example, *T. putrescentiae* been shown to contaminate various laboratory cultures, including murine facilities (Meier et al. 2009), arthropod colonies (Brust and House 1988, Papadopoulou 2006), and fungal cultures (Duek et al. 2001). Mold mites have been observed feeding on mice in research colonies (Meier et al. 2009), corn ear worm pupae (A. Morey, unpublished data, Univ. of Minnesota), southern corn root worm eggs (Brust and House 1988), cigarette beetle larvae (*Lasioderma serricorne* F.) (Papadopoulou 2006), colonies of nematodes (Walter et al. 1986), and dried Daphnia (Hubert et al. 2004). The diversity of laboratory cultures which mold mites infest illustrates a broad polyphagy.

After discovering *T. putrescentiae* infesting *Trichophyton mentagrophytes* (Priestley) colonies, Duek et al. (2001) tested which fungal cultures would provide an appropriate food source for this mite. *Tyrophagus putrescentiae* was able to establish in Dermatophyte and yeast colonies, particularly those in the genera *Trichophyton* and *Candida* (Duek et al. 2001). These mites were able to move from one colony to another, indicating that *T. putrescentiae* is highly mobile and has a high potential for dispersal (Duek et al. 2001). Another study showed *T. putrescentiae* exhibited preference for the fungi *Alternaria alternata* (Fries) Keissler and *Cladosporium cladosporioides* (Fresen.) G. A. de Vries (Hubert et al. 2004). Other fungi acting as a suitable host for *T. putrescentiae* include those in the genera: *Eurotium*, *Mycocladus*, *Penicillium*, and

Aspergillus (except for *A. niger*) (Hubert et al. 2004). The aforementioned studies show that a wide variety of fungi are suitable hosts for *T. putrescentiae*, indicating that laboratory colonies of these species may be susceptible to infestation by the mold mite. This mite has also been found in the soil, where it feeds on fungi, insects, and plant material (Smrž and Čatská 1987, Fan and Zhang 2007).

Mold mites have been suggested as a potential biological control agent, particularly of cigarette beetle larvae, as mold mite predation resulted in a 20% reduction in populations (Papadopoulou 2006). However, its non-specificity would not make this mite a promising biological control agent, as it may also infest and destroy commodities. Its ability to disperse could result in mold mite infestations in nearby commodities, as they move from a beneficial to pest status. As this mite is ubiquitous throughout nature, locating the point source of an infestation can be difficult. Mold mites have been observed in both the bird seed and dog food sections of retail stores, making it difficult to discern the original source.

Recently, mold mites were found in very large numbers infesting bagged dry (and semi-moist) dog food in grocery stores and other retail facilities in the US and other countries (Brazis et al. 2008, Canfield and Wrenn 2010). *Tyrophagus putrescentiae* may contaminate dry dog food stored in households, particularly under conditions of high relative humidity (Gill et al. 2011). The mites burrow into and consume the kibble, destroying its quality. The ubiquitous nature of this mite makes it a serious problem at any point along food production, distribution, storage, and retail. Recent infestations of mold mites in pet food aisles caused major damage to a new class of pet food. This class

included packaged semi-moist dog food of various brands, where the food "kibble" is stored in bags but the moisture content of this food exceeds 15% moisture. Concurrently, mites were found infesting bird seed in these pet food aisles, increasing the number of refugia and promoting the chance of reinfestation should the original infestation be removed (personal observation). Based on the amount of food at risk, a better prevention and control method is needed to reduce further damage and associated costs of control in food retail.

Improved understanding of the biology of this pest, including its behavior, will allow for more effective and efficient control measures and integrated pest management (IPM) programs. This information will allow for evaluation of strategies involving prevention of new infestations and reduction of current infestations. Improved prevention and control strategies will allow food manufacturers, retail facilities, and pest management professionals to develop IPM programs that address current mite infestations in retail stores. Also, improved strategies will anticipate future problems that may arise because of the variety of foods attacked by the mold mite. Improved IPM programs can be achieved by understanding the life history and biology of *T. putrescentiae*, in addition to determining the efficacy of current methods of control. Finding gaps in this knowledge will help to better control and prevent mite infestations. Gaps in knowledge have been encountered in the natural history and biology of this mite, and these gaps make it difficult to fully understand mite infestations and create effective prevention and control procedures against this mite.

Taxonomy

Tyrophagus putrescentiae is an arthropod in the Subclass Acari of the Class Arachnida, and Order Sarcoptiformes. Historically, this mite was assigned to the Order Astigmata, and this taxonomic order is often used in the literature. Like many other storage mites, *T. putrescentiae* is in the family Acaridae. (The other main families of domestic mites are Pyroglyphidae and Glycyphagidae.) Other storage mites with similar biologies to *T. putrescentiae* include the flour mite (*Acarus siro* L.), the fodder mite (*Lepidoglyphus destructor* (Schrank)), and other *Tyrophagus* spp., such as the cheese mite (*T. casei* (Oudemans)), *T. longior* (Gervais), *T. neiswanderi* Johnston & Brice, and *T. similis* Volgin. *Tyrophagus putrescentiae* has several common names, including mold mite, copra mite, and cheese mite, although 'mold mite' is most commonly used. In many cases, these common names are not unique to *T. putrescentiae*, which may result in confusion when only the common name is used. Latin name changes have also occurred at the species level, though. There was a brief change in the name to *T. communis*, but this was reverted upon appeal back to *T. putrescentiae* (Fan and Zhang 2009). All mites involved in the current study are *T. putrescentiae* based on identification using Kranz and verified independently (Barry O'Connor, Univ. Michigan, pers. comm.) Voucher specimens were deposited in the University of Minnesota Insect Collection.

Life History

The life cycle for *T. putrescentiae* consists of five stages. The translucent egg is cylindrical in shape with slightly rounded ends and an average size of 0.120 ± 0.007 mm

dia. and 0.066 ± 0.003 mm height (Callaini and Mazzini 1984). The egg is encased within two layers: an inner, homogeneous vitelline coat and an outer chorion that is covered with small, irregular mounds (Callaini and Mazzini 1984). Mold mite eggs lack aeropyles, a specialized micropylar area that is found on other mite eggs (Callaini and Mazzini 1984).

Upon hatching, a six-legged larva emerges from the egg. All remaining life stages have eight legs. *Tyrophagus putrescentiae* has two nymphal stages—the protonymph, which later molts into a tritonymph. Many stored product mites have an additional, hypopal stage (deutonymph, or 2nd instar nymph), but *T. putrescentiae* lacks this stage (Hughes 1976). After four stages, adults emerge with a typical sex ratio of 66% female (Barker 1967). Barker (1967) calculated a stable age distribution of 56% eggs, 34% immatures, and 9.7% adults, for mites reared on brewer's yeast at 13-15°C. Feeding occurs during all mobile stages of the mite. Under ideal conditions (30^{o}C, 90 ± 5 % RH), the mold mite has a generation time of 12.6 days and a population doubling time of 1.75 days (Sánchez -Ramos and Castañera 2005). Females can lay up to 24 eggs per day (at 25°C), although fecundity varies with temperature (Sánchez -Ramos and Castañera 2005). The time from egg to adult ranges from 7.2 to 106 days, depending on temperature (Sánchez -Ramos and Castañera 2001). At 25°C, the time from egg to adult is 9.4 ± 0.1 days (Sánchez -Ramos and Castañera 2001). This short generation time and high reproductive potential enables a rapid increase in infestations.

Physiological and Ecological Considerations

Mold mites are able to exploit diverse food sources and can rapidly become abundant under ideal conditions. When considering control tactics, the biology/behavior of mold mites provides information that may be helpful in developing prevention and control strategies. There is much known about these mites that should be discussed prior to considering improvements in an IPM program. However, there are some key unknowns that must be answered as well to create a more effective IPM program.

This mite is weakly sclerotized and is prone to desiccation. The mite collects moisture from the air via hygroscopic secretions from its supracoxal gland to prevent desiccation (Wharton and Furumizo 1977). High relative humidity (>65%) is favorable for *T. putrescentiae* survival and fecundity. This mite typically inhabits areas with highly variable microenvironments, including regions of high and low relative humidity. Favorable habitats often occur in specific patches that are interspersed by unfavorable areas of low humidity and an absence of food, which is highly unfavorable for mite population growth (Eaton and Kells 2009). While low humidity slows development of mites, some mites in most populations will survive, facilitating the potential for dispersal and growth when conditions become favorable (Eaton and Kells 2009).

A factor mediating dispersal from favorable sites is the alarm pheromone neryl formate, or (Z)-3,7-dimethyl-2,6-octadienyl formate (Kuwahara et al. 1975, Kuwahara 1982). Neryl formate is secreted from the opisthonotal gland (Morino et al. 1997). The alarm pheromone is species-specific and stimulates escape behavior at 10 ppm (Kuwahara et al. 1989). Kuwahara (1982) tested the ability of different monoterpenoids

and related chemicals to function as an alarm pheromone for *T. putrescentiae*. This involved the synthesis of compounds with different functional groups, including (Z)- and (E)-isomers of each compound; the natural pheromone was also used (Kuwahara 1982). All (E)-isomers were inactive and the (Z)-isomers had differing levels of activity (Kuwahara 1982). Presence of a (Z)-allylic primary alcohol moiety was necessary for developing alarm pheromone activity in *T. putrescentiae*, and formylation of the hydroxy group improved the activity (Kuwahara 1982). This determined the structures that are important in inducing alarm pheromone activity in *T. putrescentiae* and showed that synthetic neryl formate works similarly to the naturally-derived pheromone (Kuwahara 1982). What is unknown about neryl formate is its influence on dispersal when population density and humidity are taken into consideration. Specifically, is there a possible interaction between population density and humidity and the concentration of neryl formate?

At some point, mite density in favorable patches will exceed the allowable area, and possibly the carrying capacity, and the mites will disperse in search of new favorable patches. This inter-patch movement may be across areas of low humidity. While the mite is prone to desiccation, it can survive short periods of time in areas of low humidity. "Explosive dispersal" has been detected at relative humidities as low as 15% (Eaton and Kells 2009; Kells pers. comm.) Explosive dispersal behavior is characterized by sudden movement from a point source of large numbers of a species over a short period of time (Aylmer and Reisner 1971). In the case of mold mites, this time period is about 24-48 hours. During this dispersal, mites can avoid desiccation from low humidity by clumping

together (Sánchez-Ramos et al. 2007).

While explosive dispersal has been observed, it is unknown if this type of dispersal behavior is impacted by humidity, or if explosive dispersal is the sole dispersal mechanism of this mite. Conceivably, there may be a bimodal dispersal strategy where constant emigration occurs at high humidity and explosive emigration occurs at low humidity, and if so, it is important to define the critical humidity at which the dispersal behavior would change. Also, the possible interaction between mite density and humidity and how it may affect dispersal may be crucial in understanding the population dynamics of *T. putrescentiae*, infestation patterns, and feeding damage. Learning more about the dispersal behavior and the influence of population dynamics will provide an understanding into better ways of prevention and control of this pest. Understanding dispersal behavior will permit more complex studies involving the effects of neryl formate.

Dispersal behavior of *T. putrescentiae* away from an enclosed food source may be affected by relative humidity outside the food container. At optimal humidities, mites have been shown to freely move away from and back to the food source (unpublished data). It is unknown whether this dispersal occurs via continuous dispersal or explosive dispersal. Continuous dispersal involves constant movement of mites from an infested bag and a slow accumulation of mites in the shelving. Under explosive dispersal, most of the mites stay in the infested source until resources become limited and then move in large numbers over a short period of time (12-24 hours). Dispersal behavior of *T. putrescentiae* away from an enclosed food source may be affected by relative humidity

outside of the food container. Additionally, there may be impacts on the ability to detect this pest if they are not continuously dispersing, as monitoring methods only detect mites moving out of the food source.

Types of competition may also affect movement patterns of this mite. There are two types of competition: scramble and contest (Santos 1989). In scramble competition, individuals are not affected by one another with their ability to acquire nutrition, but the difficulty in finding nutrition results from a depleted resource and difficulty finding the food resource (Santos 1989). In contest competition, which has been shown to be the primary mechanism of competition at high densities of *T. putrescentiae*, the mite individuals compete for food source (Santos 1989). This means that all individuals have access to the food source, but some individuals interfere with the ability of others to acquire nutrition from the food source. Which type of competition occurs may be a result of population density (Santos 1989). In Santos' (1989) experiment, adults were competing for space on the food source, which reduced in size over time as it was consumed, thus continuing to push away individuals that had originally acquired food. As long as females remained on the food source long enough to get enough nutrition to reproduce, they produced eggs (Santos 1989). One granule of Fleischmann's yeast (approximately 200 μg) could feed 20 adult mites at once. Mites that arrive at the granule after the first 20 individuals arrived were unable to get to the food, thus illustrating contest competition (Santos 1989). Understanding the types of competition may provide information about mite dispersal and the stimuli that cause mites to disperse.

Chemo-orientation may play a role in mold mite behavior, as mold mites may respond to chemical cues in their environment. Y-tube olfactometer tests showed that *T. putrescentiae* responded to arms containing humidified air and also responded significantly to arms containing fish flake volatiles (Skelton et al. 2007). This shows that mites are attracted to humid air, but may also be attracted to food volatiles, which may make it easier for them to locate a food source. The mechanism of detection of these food volatiles is unknown.

Mites can survive over a wide range of temperatures. Adult and nymphal mold mites are freeze intolerant: the lower lethal temperature LLT_{90} for adult and nymph mold mites occurs above the supercooling point (SCP) (Eaton and Kells 2011). Mold mite eggs, however, are freeze tolerant, so some mite eggs may survive freezing and later hatch (Eaton and Kells 2011). Freeze survival of eggs occurred as low as -18°C, and the estimated LLT_{70} for eggs is 35.0°C (-44.0, -29.7) (Eaton and Kells 2011). Estimates for the upper temperature limits for mold mites range from 35.5°C for larvae to 37.4°C for eggs, based on survival data from 10-34°C (Sánchez-Ramos and Castañera 2001). These numbers indicate that the mites are fairly heat tolerant, although temperatures above 30°C result in a decrease in mite longevity (Sánchez-Ramos and Castañera 2001, Sánchez-Ramos and Castañera 2005). However, as relative humidity is inversely dependent on temperature, it is unknown if the high temperatures or the precipitous decrease in relative humidity is the main mode of injury.

Integrated Pest Management

Prevention and Control Methods

Many pest management tactics can be used to prevent and control mite infestations and be combined into an Integrated Pest Management (IPM) program, providing a strategy to avoid infestations and minimize impacts on food products. Understanding the behavior, preferred habitat, and potential food sources of the pest allows for a better targeted IPM strategy. An IPM program utilizes several steps to increase the likelihood of success in prevention and control of infestations, including product rotation, sanitation, exclusion, inspection, monitoring, and chemical and non-chemical controls. Exclusion methods include the use of barriers to prevent the spread of the pest species, and these barriers may be chemical or non-chemical. In order to be effective, an IPM program needs to take into account the business constraints and attributes that may affect development of prevention and control practices. Unfortunately, during early assessments in retail facilities, it was found that narrow economic margins, lack of understanding of pest prevention, and blaming suppliers for any pest problems resulted in retail managers seldom taking ownership of a pest issue. Inspections for early detection of infestations were lacking, product rotation seldom occurred, and sanitation was limited to cosmetic/aesthetic or superficial cleaning. Current knowledge and business interests affect the use and extent of IPM practices. Each practice should be summarized for its potential utility against this recent increase in infestations.

Product Rotation

Product rotation ensures that products are moved from the store in the order in which they arrived. First in/first out (FIFO) is a standard practice where new product is placed behind older products to ensure that all products are moved out of the store before expiring. If products remain on the shelf and are located by founding mites, an infestation can initiate and rapidly build up. Once this occurs, dispersal of mites will spread the infestation to other products. Unfortunately, product rotation is limited or non-existent in many pet food aisles. Instead of moving new product to the back of the shelves, new product is often stocked in front or on top of old product, resulting in extended storage of food packages and stable habitats for mite development. Packages showing their age on the shelf result in consumers picking up newer packages, so the infestation continues. If product rotation continues to be a problem, a sizable infestation can build, and mites, due to their small size, remain unnoticed until the infestation becomes extreme.

Sanitation

Sanitation, which includes cleaning of shelving and floors and removal of spilled food on and under shelving using a broom or vacuum, is theoretically important in controlling mold mites. However, pest source reduction must be accomplished in addition to sanitation measures. One study indicated that a single thorough sanitation did not significantly reduce the number of beetle pests in retail stores (Nansen et al. 2004). This indicates that sanitation may need to be repeatedly performed in order to effectively reduce infestations of stored product insects and mites (Nansen et al. 2004). While a

superficial cleaning for aesthetic purposes is often done, food and moisture are still left behind and under shelving, which can provide multiple refugia for mold mites. A superficial cleaning temporarily improves the aesthetics/appearance of the shelving by removing visible infestations; however, the infestations still persist inside the bags and will likely move out onto the shelving again.

Exclusion

Two kinds of exclusion exist for use against mites: physical or chemical. Certain types of packaging can prevent mites from entering and consuming the food and can kill mites already in the food, thereby protecting the commodity. Modified atmosphere packaging (MAP) increases the carbon dioxide content in the packaging using a mixture of gases and a vacuum packaging machine, which make the package unfavorable for the growth or survival of various pest species (Riudavets et al. 2009). Unfortunately, this type of packaging is not practical for use in dog food bags, as it makes the food more difficult to transport and becomes ineffective if the packaging is broken. In order to make dog food packages easy to transport and handle without damaging or taking up too much space, the packaging needs to allow gas exchange. Gas permeable packaging is more suitable for mite development than vacuum-sealed packaging. Therefore, modified atmosphere packaging is more practical for small packages and/or packages that are handled very little.

If other prevention or exclusion methods are not possible or are limiting, barriers are an option that may be considered to prevent access of pests to susceptible food. Barriers may be physical, such as changes in packaging, or chemical, in the form of

acaricide applications. Monitoring traps only detect mites that have left their infestation sources, and it is unknown whether the mites leave infested bags at a continuous rate or if mites are more likely to leave as populations explode and food becomes limiting.

Residual acaricides applied to floors, support posts, and shelf backing may provide a chemical barrier to infestations by preventing mites from traveling to and from refugia and shelves. However, use of residual acaricides as a barrier to mite dispersal needs to be tested. It may be difficult to control mites in microhabitats, so a barrier exclusion method may be useful in preventing the mites from dispersing from microhabitats to susceptible food sources. Residual acaricides, also a form of chemical control, applied to floors, support posts, and shelf backing may act as a suitable barrier against dispersal from microhabitats to food packaging. This will also prevent dispersal of mites between shelves. Using a barrier method to isolate infestations is also important, because of the difficulty in detecting infestations with current monitoring methods.

Inspection

Inspections involve examining the shelves for damaged, old, or infested packaging, so they can be removed. In retail habitats, inspections are superficial and seldom completed. Unfortunately, most retail employees are not trained in identifying mold mites, and infestations are often overlooked until these infestations produce a thick layer of biomass. This biomass consists of live and dead mites, frass, and exuviae and resembles dust on the bags and shelving. Store employees may clean up this "dust" without realizing it is a sign that there are infested food packages on the shelving.

Monitoring

Mite monitoring is often done by placing baited traps around the shelving in the dog food aisle for 24 to 48 hours and inspecting the traps for mites (Dunn et al. 2005). Traps with a higher number of mites are more likely nearer to the source of the infestation, so use of traps may be helpful in identifying locations of infestations. Unfortunately, many stores do not have monitoring programs to detect mold mite infestations, so infestations may go unnoticed for long periods of time. Monitoring for only 24 or 48 h is a labor-intensive process resulting in substantial costs to the IPM program. Costs can be reduced by having on site personnel conduct the monitoring, but training and equipment to view the mites still makes monitoring cost prohibitive.

In urban IPM, monitoring usually occurs on a monthly basis where traps are checked during the regular pest management service. Mite monitoring is not practical in this retail situation and a lack of monitoring lessens the effectiveness of an IPM program. In addition to the impracticality, there is also the issue of mite dispersal characteristics, which may influence the efficacy of monitoring procedures.

Recent vacuum sampling in a newly established pet food store indicated that mite dispersal may occur up to 50 ft (15.24 m) from a point source within two weeks (Kells unpublished data). This dispersal potential requires traps to be positioned widely in an area. How extensive an infestation must be prior to trap detection is an issue. Preliminary observations indicate that only a few mites leave the food source until populations have reached high numbers and food resources have been depleted. The mites seem to leave the infestation site in large numbers and accumulate on shelving in a

short period of time (24-48 hours), which may be a sign of explosive dispersal. Since infestation levels may be relatively high inside the food packaging before many mites leave, there may be low levels of mites found in bait traps, even when there is a high-grade infestation on the shelves. This makes monitoring difficult, thus making control difficult, as high levels of infestation may persist with little detection from monitoring procedures. This further complicates control efforts, as infestations may go unnoticed for long periods of time and then "suddenly" appear.

Chemical and Non-chemical Controls

Various chemical and non-chemical control methods can be utilized to reduce mold mite population numbers. Chemical control methods include the use of acaricides to kill the mites. Non-chemical methods include physical methods, such as changes in temperature and humidity, to kill mites by creating an unsuitable environment for the mites. These methods can be used separately or together to control mold mite infestations.

Chemical control methods are used in stored grain and other unfinished food products (Stará et al. 2011). While chemical control methods may seem like a quick and useful solution, there are restrictions to the types of acaricides that can be used near finished food products (Stará et al. 2011). There is evidence for the acaricidal efficacy of botanical extracts and synthetic compounds against *T. putrescentiae*. However, many of the compounds that have been tested were tested on porous surfaces, such as cotton or paper disks (Kim et al. 2003, Tak et al. 2006); infused into the food sources (Hubert et al. 2007); used as inert dusts on the raw commodity itself (Palyvos et al. 2006); or used as

fumigants (Sánchez-Ramos and Castañera 2001). In retail facilities, residual acaricides are a more practical application than fumigation, as the acaricide remains for an extended period of time and product handling procedures are greatly reduced. An advantage to natural/botanical acaricides is that their labels tend to be less restrictive, so a more extensive application can be made to shelving, providing for a wider barrier between possible mite sources and susceptible food products.

In addition to chemical control methods, changes to the environment in which the mold mites live can cause mortality or limit population growth. Low humidity restricts mite development, so monitoring and controlling humidity allows for a reduction in potential mite activity (Eaton and Kells 2009). However, humidity is often difficult to control, especially in small, secluded areas like under shelving and behind kick plates. Areas of high relative humidity in pet food shelving make it difficult to prevent and control mold mite infestations. Additionally, relative humidity plays a role in mite survival and could affect acaricide efficacy regardless of the mode of action, and this has been documented for desiccants (Cook and Armitage 2000). Diatomaceous earth formulations are commonly used to control mites that infest grains and stored products (Collins and Cook 2006, Palyvos et al. 2006).

Ultraviolet (UV) radiation, another method of non-chemical control, can be lethal to mold mites. While lethality can occur from UV damage, occasionally the mite is able to repair damage using photoreactivity (Santos 2005, Collins and Kitchingman 2010). Photoreactivity involves returning DNA to its normal state by splitting the dimer that was formed due to UV light exposure (Santos 2005). Ultraviolet B radiation exposure for one

hour at 1300 μW/cm^2 resulted in 100% death of *T. putrescentiae* (Santos 2005). Santos (2005) found no difference between the means of death rates after exposure to UV-B of mold mite populations exposed to visible light and those kept in the dark. Collins and Kitchingman (2010) studied the effects of UV-C radiation at 254 nm and 9 mW/cm^2 at a distance of 9 cm, on *T. putrescentiae*, including death rates on different stages of mites and sublethal effects, including those on mite development and fecundity. This study found that the average mortality (ED_{50} and ED_{95} (μJ/cm^2)) of UV-C radiation was significantly different in mold mites raised in dark compared to mold mites incubated in the light (Collins and Kitchingman 2010). These results conflict with the findings of Santos (2005), although a different type of UV radiation, with a shorter wavelength, was used. The study found no significant difference in number of progeny between mites in the control and mites treated with UV-C radiation for 12 s, although the authors noted that in many replicates a majority of the females died during the incubation period before laying eggs, so further research is warranted (Collins and Kitchingman 2010). Also noted was that dust and debris tended to limit the amount of radiation absorbed by the mite, so sanitation may be important in increasing efficacy of radiation treatments (Collins and Kitchingman 2010). The aforementioned studies indicate that the use of ultraviolet radiation may be useful in managing exposed mold mites, although its limitations indicate that it is best used in combination with other practices.

Several mite species can feed on *T. putrescentiae*, thus serving as a potential method of biological control. Many of these mites are in the family Cheyletidae, including *Cheyletus malaccensis* Oudemans and *Cheyletus eruditus* (Schrank), although

mites from other families have been shown to feed on *T. putrescentiae* (Enkegaard et al. 1997, Palyvos et al. 2006, Ždárková 1995). The predatory mite *Hypoaspis miles* (Berlese), a polyphagus mite, has been shown to successfully feed and reproduce on a diet of *T. putrescentiae* (Enkegaard et al. 1997). Studies show that *C. eruditus* is less susceptible to organophosphates than *T. putrescentiae* (Ždárková 1995), and *C. malaccensis* is less susceptible to diatomaceous earth than *T. putrescentiae* when applied to stored grains (Palyvos et al. 2006). These results indicate that concurrent chemical and biological control may be possible in an integrated pest management program against mold mites in unfinished food products (Ždárková 1995, Palyvos et al. 2006), but are of limited application in the retail habitat.

Conclusion

While there has been research done on the basic biology of *T. putrescentiae*, including abiotic factors affecting survival (upper and lower temperature and relative humidity limits), there are several aspects about the biology and management of this pest that require additional research. For example, increased knowledge about the dispersal behavior of mold mites will allow for improved understanding of the effectiveness of monitoring methods and estimations of infestation level. Understanding the efficacy of residual acaricides against the mold mite will allow for an effective exclusion method to prevent mites from moving between microhabitats. The indoor retail habitat is unique in that it has no seasons, thus potentially providing conditions favorable for continuous mold mite population growth, and managing mold mites is exacerbated by the few

chemicals labeled for use near finished food products. This project addresses two critical elements of IPM: improved understanding of pest behavior, and the use of selected acaricides as a barrier to mold mites. If we understand these additional aspects of mold mite biology, then we can develop a better targeted IPM program against mold mites in retail facilities.

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Chapter 2. Efficacy of selected residual acaricides against the mold mite, *Tyrophagus putrescentiae* (Acari: Acaridae)

Summary

The mold mite, *Tyrophagus putrescentiae* (Schrank), is a stored product pest of economic significance that commonly infests many types of food and animal feed products. There is limited information regarding pest management tactics, including residual acaricides for managing this pest. The purpose of this study was to assess the potential of selected acaricides that could provide a protective barrier for susceptible food products, particularly in retail stores near food packages destined for consumer use. This study was designed to assess the efficacy of commercially available, residual acaricides that would be compatible with retail operations. Mite mortality after 24 and 48 hours was measured after exposing mites to acaricide residues applied to a nonporous surface. Two additional factors included in the study were humidity and the presence of an adjuvant to facilitate spread of acaricide on nonporous surfaces. Increasing humidity levels generally decreased acaricide efficacy. Use of the adjuvant itself did not appear to affect efficacy, but the type of application (wet/slurry or dry) of dust acaricides significantly affected efficacy. Residual acaricides may act as an effective barrier (within 24 – 48 h) against dispersal of this mite from one infestation site, or refuge, to another. Future work should examine sub-lethal effects of acaricides, including the behavior of intoxicated mites.

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Introduction

The mold mite, *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae), is a stored product pest of economic significance that is a problem in many types of food. This mite can cause damage to grains (Hughes 1976), cheese (Robertson 1952), cured ham (Arnau & Guerrero 1994), and pet food (Brazis et al. 2008). In addition to destroying food, there is evidence that the mite may be a source of allergens affecting dogs (*Canis lupus familiaris*) and humans (*Homo sapiens* L.) (Mueller et al. 2005).

Mold mites may be found at several points along the food storage, processing, and distribution system, including raw grain through to finished food products. Once a mite outbreak has been detected, it can be difficult to fully control the infestation. Recent infestations have been found in bagged dry (and semi-moist) dog food in grocery stores and other retail facilities in the US and other countries (Brazis et al. 2008, Canfield and Wrenn 2010), as well as in households (Gill et al. 2011). Damage to pet food occurs when the mites burrow into and consume the kibble, thus destroying its quality. Mites that disperse from an infestation can hide on and around shelving and in cracks and crevices on the floor under the shelving. Recent vacuum sampling in a newly established pet food store indicated that mite dispersal may occur up to 15.2 m from a point source (Kells unpublished data). This dispersal potential allows for reinfestation of new food or feed products, even after the initial infestation has been identified and removed.

While mold mites can infest commodities at any point along the food production, grain storage, food processing, and distribution system, including infestation of the finished products, the pest management procedures may greatly differ depending on

which point in the process the infestation occurs. For example, agricultural and industrial facilities may use controls such as fumigation, and acaricidal sprays. In retail facilities, fewer control options are available. Product dis-infestation via fumigants is not practical or available and there are limitations as to the deployment of spray acaricides around finished product packages. The principal control method available in retail stores is disposal of infested packages. Sanitation is important in controlling infestations by reducing food and harborage sources for the mite, in the retail environment. However, the complexity in retail shelving makes it difficult to use sanitation as an effective control and prevention method. The use of residual acaricides that are compatible with retail operations and in combination with sanitation may provide a barrier to prevent dispersal of this mite between refugia that cannot be addressed by sanitation measures and finished food product.

There is limited information regarding the efficacy of residual acaricides that would help provide a protective barrier against mites attacking susceptible food products in retail habitats. With other stored product pests, particularly *Tribolium castaneum* (Herbst), residual insecticide barriers have been shown to decrease trap catches in warehouses, (Toews et al. 2009), hence the number of pests entering an area protected by a residual insecticide. In retail facilities, residual acaricides have a more practical potential than fumigation, as residual acaricides remain for an extended period of time and product handling procedures are greatly reduced. Also, most shelving material is nonporous in design which favors exposure of the mites to active residues. An advantage to natural/botanical acaricides as residual products is that available product labels tend to

be less restrictive than synthetic acaricides, so a more extensive application might be applied to shelving, providing for wider barrier between possible mite sources and susceptible food products.

A challenge to the use of residual acaricides in retail situations is the restrictions to the types of acaricides that can be used near finished food products (Stará et al. 2011) and the current knowledge pertaining to acaricide efficacy on nonporous surfaces. While there is evidence for the acaricidal efficacy of botanical extracts and synthetic compounds against *T. putrescentiae*, many of the compounds that have been tested were tested on porous surfaces, such as cotton or paper disks (Kim et al. 2003, Tak et al. 2006); mixed into the food sources (Hubert et al. 2007, Hubert et al. 2008, Gulati and Mathur 1995); used as inert dusts on the raw commodity itself (Palyvos et al. 2006); or used as fumigants (Sánchez-Ramos and Castañera 2001). Ascertaining the efficacy of formulated acaricides against on nonporous surfaces is important for further recommendations pertaining to acaricidal barriers to protect finished products on retail shelves.

Mold mites are subject to stresses from variable humidity in the habitat, and humidity is another potential factor influencing acaricide efficacy. *Tyrophagus putrescentiae* mites are weakly sclerotized, so they are prone to desiccation (Sánchez-Ramos et al. 2007). High relative humidity (>65%) is favorable for *T. putrescentiae* survival and reproduction/fecundity, and under ideal conditions (30 °C and 90 ± 5% RH) mites have a generation time of 12.6 days and a population doubling time of 1.75 days, so they can quickly reach very large densities (Sánchez-Ramos and Castañera 2005). The

biology of this mite favors desiccation is an effective mode of action when selecting an acaricide and relative humidity could affect acaricide efficacy regardless of the mode of action (Cook and Armitage 2000).

The objective of this study was to determine efficacy of selected residual acaricides against *T. putrescentiae*, with an emphasis on products that are commercially available and compatible with retail operations. We also evaluated acaricides that are often used by commercial pest management contractors in efforts to manage mold mites in retail stores. In the retail habitat, relative humidity is variable with areas that are both favorable and unfavorable for mite development. We therefore included a range of humidity levels in evaluating acaricide efficacy.

Materials and Methods

Mite Rearing and Arena Design.

The mold mite, *T. putrescentiae*, was reared in 0.946 L Mason® jars on semi-moist dog food (Eaton and Kells 2011). Jar lids were replaced with mesh screen with filter paper (P5, Fisher Scientific) on both sides of the screen to provide ventilation. These modified lids were held on by securing the band on the screw threads. The jars were placed in larger plastic containers with water to maintain high humidity. Jars were incubated at room temperature (22-25°C). The lids of the larger containers had 4 holes (1.3 cm dia.) for ventilation.

Arenas consisted of polystyrene Petri dishes (50 mm x 9 mm, Falcon®, Becton Dickinson Labware, Franklin lakes, NJ). A 9.7 mm dia. holes was punched in each lid

and mesh (Precision Woven Nylon Mesh, 193 X 193 Mesh, 0.07874 mm opening, McMaster-Carr, Chicago, IL) was adhered over the holes with hot glue (Arrow all purpose clear glue sticks, Saddlebrook, NJ) to provide ventilation and prevent escape. Arenas were sprayed with assigned acaricide treatment (Table 1) to the point of runoff and allowed to dry.

Mites were transferred via nylon paintbrush from Mason® jar lids to treated arenas. Ten (10) unsexed adult mites were transferred to each treated arena, and the dishes were placed into humidity chambers, where they were allowed to incubate at room temperature for approximately 48 h. Mortality was recorded, and the Petri dishes were placed back into the humidity chambers. Mortality was recorded at 24 and 48 h. Mites were considered dead if they were desiccated or exhibited no movement when agitated with a paintbrush hair.

Saturated salts were used to maintain consistent relative humidity levels in plastic desiccators (Winston and Bates 1960). Saturated salts were used to provide 3 relative humidity levels at room temperature: $NaNO_3$ (68% RH), NH_4NO_3 (64% RH) and $MgCl_2$ (40% RH). Relative humidity levels were measured daily with a portable temperature/RH meter (Vaisala HMI41 with a HMP42 probe; Helsinki, Finland) to confirm average relative humidity.

Exposure Procedures: Experiment 1

Assembled Petri dish arenas were pre-weighed and randomly assigned to a treatment group. Arenas were numbered and the inside of each arena was covered with the assigned treatment to the point of runoff and excess fluid was tapped off. After plates

Table 1. Characteristics of selected residual acaricides used in the study

Acaricide	Lot Number	Active Ingredient(s)	Dilution rate
EcoAdjuvant®		2,6,8 - Trimethyl-4-nonyloxypolyethyleneoxyethanol, 50.00%	7.81 ml/L
EcoExempt® D[1]	90213001	2-Phenethyl Propionate 4.50% Soybean Oil 2.00% Eugenol (Clove Oil) 1.75%	2%, dilution of whole powder
EcoExempt® IC[2] [1]	08249/8I01	Rosemary Oil 10.00% Peppermint Oil 2.00%	46.875 ml/L
EcoPCO® DX[1]	60328001	2-Phenethyl Propionate 1.00% Pyrethrins 0.40%	2%, dilution of whole powder
EcoPCO® WPX	246082	2-Phenethyl Propionate 3.00% Thyme Oil 5.00% Pyrethrins 0.50%	0.0125% solution
Mother Earth™ D[1]	270005 07025	Diatomaceous Earth (including silicon dioxide, other oxides and moisture) 100.00%	2%, dilution of whole powder
Talstar® P	M8O3-002	7.90% Bifenthrin	0.062% solution
Tempo Ultra® WP	9-98-9002 JIO64	10.00% Cyfluthrin	0.05% solution
Water (control)	N/A	N/A	100%

[1]- EcoAdjuvant was added to these solutions to promote mixing in the water during the first experiment.

were dry, they were reweighed to determine mass of residue that adhered to the plates. The plates of each treatment were randomly assigned to one of three buckets, and each bucket was randomly assigned to one of 3 humidity levels. There were 9 treatment groups with 6 replicates for each acaricide-humidity combination.

Selected acaricides included EcoExempt D, EcoExempt IC^2, EcoPCO DX, EcoPCO WP, Mother Earth D, Talstar P, and Tempo Ultra WP. These acaricides were mixed with deionized water. Being dusts, EcoExempt D, EcoPCO DX, and Mother Earth D were diluted to 2% of the whole powder as per calculations to determine the amount of dust applied to the surface according to label rate without water. The solutions also contained EcoAdjuvant (7.81 ml/L) to facilitate mixing and even spread of acaricides on plastic or metal surfaces. EcoAdjuvant was also used with EcoExempt IC^2 to form an emulsion. Solutions were applied to both the base and the lid of the Petri dishes and swirled around to provide an even coating. Control dishes received deionized water as a treatment. Excess solution was tapped off and the dishes were allowed to dry for at least 24 hours.

Exposure Procedures: Experiment 2

A second experiment tested the influence of the adjuvant on efficacy of the dust acaricides. Treatments included the EcoAdjuvant, EcoExempt D, EcoPCO DX, and Mother Earth D. The trials involved: 1) dusts as a dry application only, 2) adjuvant applied and dried, followed by a dry dust application, and 3) adjuvant and dust mixed into an aqueous solution. There were a total of 11 treatment groups with 6 replicates and 10 adult mites per replicate. Solution concentrations were the same as in Experiment 1.

Relative humidity levels used were 64 and 68% RH. Exposure procedures were the same as Experiment 1, except the controls were untreated, no repeated measures were used and proportion mortality was measured at 48 hours.

Statistical Analysis

Proportion mortality data were transformed using Arcsin(Sqrt(x)) and differences were determined using SAS Proc GLM with brand and humidity as class variables (SAS Institute 2009). Protected comparisons using the transformed means (proportions ± SE) were performed via Least Squares Means ($p = 0.05$). Comparisons were restricted between the treatment and the corresponding control. Mean mortality (±95% CI) was represented graphically to illustrate differences.

Results

Experiment 1

Mean percent mortality was affected by acaricide, humidity, and the interaction between acaricide and humidity ($F_{16,135}=2.47$, $p<0.05$) (Table 2). With the interaction significant, evaluation of results required that the simple effects be evaluated within each humidity level. Decreasing humidity increased the effectiveness of nearly all acaricide treatments (Figs. 1-2). Compared to the controls, significant efficacy occurred within 24 hours with EcoExempt D, EcoPCO DX and Mother Earth D (Fig. 1). Dusts caused dead mites to appear desiccated, with a shriveled appearance and their legs curled. At 24 hours, EcoExempt IC^2, an emulsion, was effective at all three humidities, and dead mites in this treatment had a bloated and glossy appearance (Fig. 1). Dead mites in the

Table 2. Summary of analysis of variance for factors affecting efficacy of selected residual acaricides against mites. The factors tested included humidity and acaricide treatment. Efficacy was recorded at 24 and 48 h, hence the factors were evaluated for both of these times.

Effect/Source	DF	Sum of Squares	Mean Square	F value	Pr >F[2]
		24 h			
Model	26	17.18637	0.66101	8.65	<0.0001
Treatment	8	12.66234	1.58279	20.70	<0.0001
Humidity	2	1.30413	0.65207	8.53	0.0003
Treatment*Humidity	16	3.21990	0.20124	2.63	0.0013
Error	135	10.32048	0.076448	-	-
Corrected Total	161	27.50685	-	-	-
		48 h			
Model	26	30.39188	1.16892	13.51	<0.0001
Treatment	8	13.84692	1.73087	20.00	<0.0001
Humidity	2	13.35555	6.67778	77.17	<0.0001
Treatment*Humidity	16	3.48907	0.21807	2.52	0.0021
Error	131	11.33600	0.086534	-	-
Corrected Total	157[1]	41.72788	-	-	-

[1] - Four arenas were excluded from statistical analysis due to a low count of mites present. These mites may have escaped.

[2] - Significance was measured at $p<0.05$.

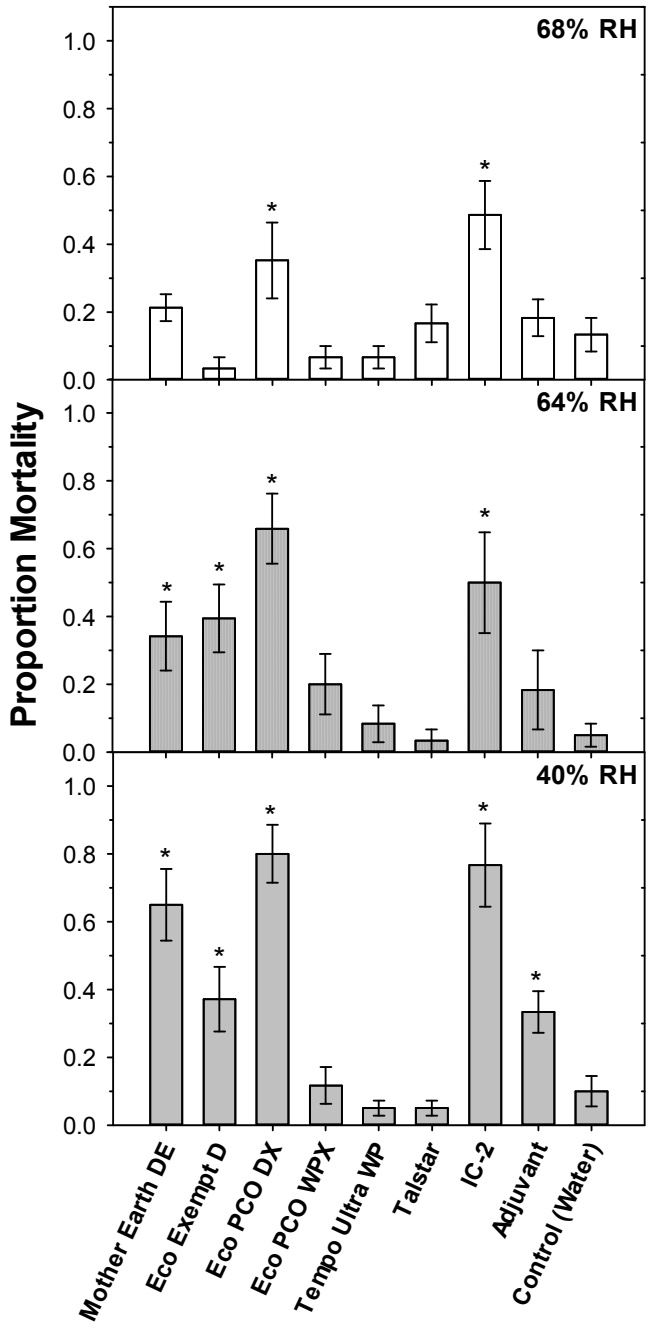

Figure 1. Mean proportion mortality of *T. putrescentiae* 24 h after exposure to selected acaricides, at three relative humidities. Asterisks (*) indicate the treatment was significantly different from the control ($p<0.05$). Because of the interaction, comparisons were limited to within humidity differences.

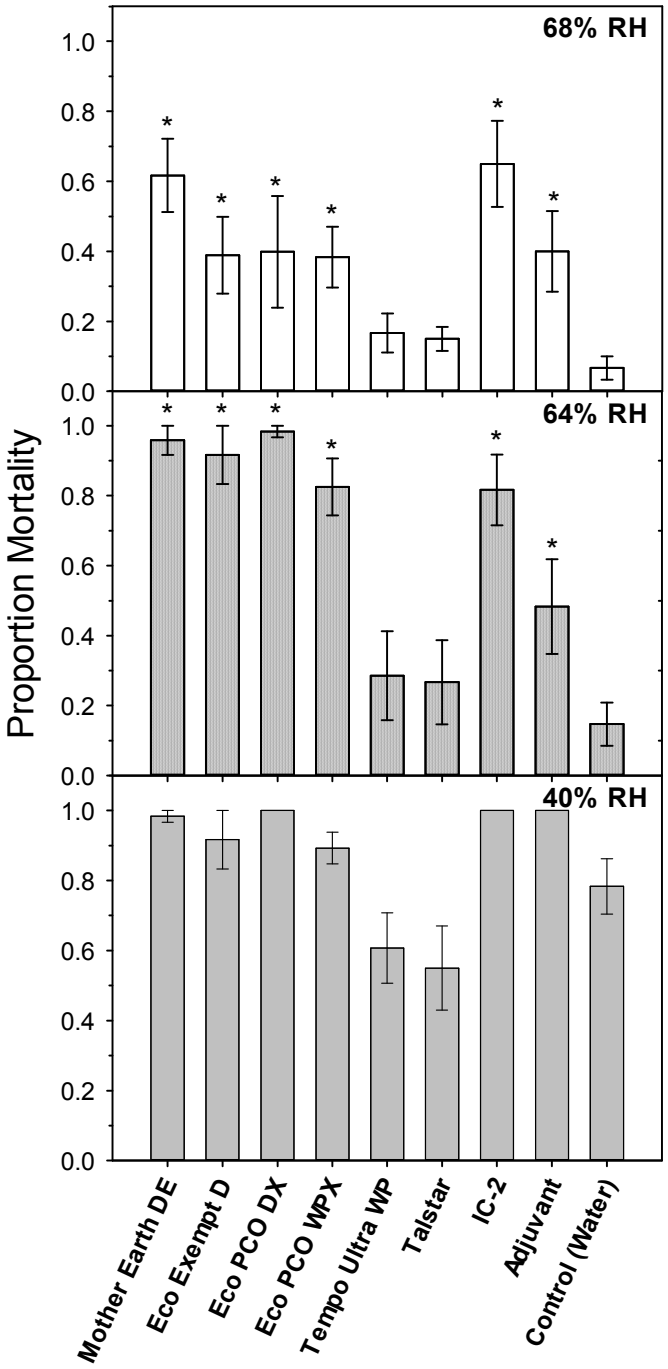

Figure 2. Mean proportion mortality of *T. putrescentiae* 48 h after exposure to selected acaricides, at three relative humidities. Asterisks (*) indicate the treatment was significantly different from the control ($p<0.05$). Because of the interaction, comparisons were limited to within humidity differences.

adjuvant treatment also had a glossy appearance, but were not bloated. This trend continued at 48 hours, except that more products showed efficacy compared to the control (Fig. 2). Mortality in the controls approached 80% in the low humidity conditions. In this study, diatomaceous earth and the Eco products showed additional efficacy greater than controls, while synthetic pyrethroids showed little residual efficacy.

Experiment 2

As the adjuvant (EcoAdjuvant) itself showed efficacy in the first experiment, the second experiment analyzed the application type of the acaricide. Application types included the effect of wet and dry dust applications in the presence of an adjuvant, at humidity levels that are favorable for mite survival (64 and 68% RH). At 48 hours, the acaricide treatment and the interaction between acaricide and humidity were significant ($F_{10,109}$=2.46, $p<0.05$) (Table 3), and most treatments were more effective than the control (Fig. 3). However, the dust formulated with water and adjuvant had decreased efficacy compared to the treatments where adjuvant and dust were applied separately. Wet applications of EcoExempt D were not significantly different from control, whereas wet application of EcoPCO DX was not significantly different from adjuvant only application (Fig. 3). At 68% relative humidity (RH), conditions were more challenging for acaricide efficacy, and for simplicity we only show these results. However, mortalities showed a similar pattern at a more marginal humidity of 64% RH to those at 68% RH (Fig. 3). Dry-plus-adjuvant and dry-only applications were not significantly different from each other within each acaricide brand.

Table 3. Summary of analysis of variance for factors affecting efficacy of selected residual acaricide applications against mold mites. The factors tested included humidity and acaricide application treatment. Efficacy was recorded at 48 h.

Effect/Source	DF	Sum of Squares	Mean Square	F value	Pr >F[1]
Model	21	29.29019	1.39477	24.28	<0.0001
Treatment	10	27.96928	2.79693	48.69	<0.0001
Humidity	1	0.12712	0.12712	2.21	0.1399
Treatment*Humidity	10	1.26328	0.12633	2.20	0.0235
Error	102	5.85904	0.057442	-	-
Corrected Total	123	35.14923	-	-	-

[1] - Significance was measured at $p<0.05$.

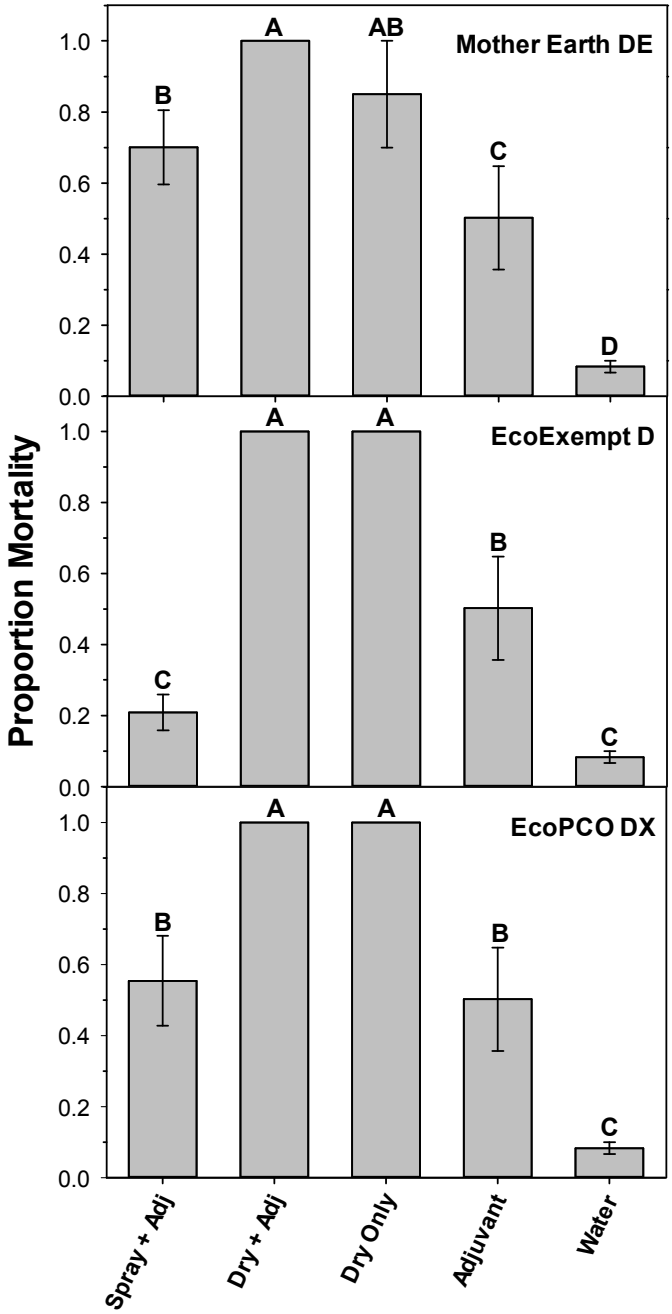

Figure 3. Mean proportion mortality of *T. putrescentiae* 48 h after exposure to selected acaricides at 68% RH. Comparisons were performed within each acaricide; a different letter indicates statistical significance ($p<0.05$). Treatments included dusts as a dry application only; adjuvant applied and dried, followed by a dry dust application; and adjuvant and dust mixed into an aqueous solution.

Discussion

The mold mite is a persistent pest in some retail pet food displays in North America, which currently necessitates the use of commercially available acaricides approved for use around finished food and animal feed displays. We define such retail products to be at-risk if they provide food and humidity suitable for growth and development. In the retail store habitat, there are a plethora of refugia where this mite may exist and maintain active infestations, such as in packaged food and other peripheral areas. Peripheral areas can be quite diverse and include: under flooring tiles; expansion joints or stress cracks in concrete flooring; cracks, crevices and seams on shelving; and where the shelving structure contacts the floor. Abundant refugia complicate efforts to control this mite by sanitation and exclusion practices alone. Breaking the cycle of infestation involves removal of infestation source(s) and the creation of a barrier between mites in refugia and at-risk products. The present study tested candidate acaricides that are commercially available, and might be effective in creating a barrier against *T. putrescentiae* in retail conditions. Of the acaricides used in this experiment, only three currently include "mites" on the label: EcoExempt IC^2, Mother Earth D, and Talstar P. The other products had labels compatible with use within retail settings, but had not been previously evaluated for efficacy against *T. putrescentiae*.

For residual acaricide applications to act as an effective barrier against *T. putrescentiae*, they should cause efficacy within at most 48 hours regardless of humidity. Efficacy within this time period would prevent dispersal of mites from refugia toward new food sources. The time frame 24-48 hours was selected theoretically based on

mite's potential distance of dispersal. Short term travel velocities for this mite have been estimated at 135-227 cm/ h (unpublished data). This indicates a high capacity for mite dispersal, and, therefore, significant mortality due to the acaricide should be achieved as quickly as possible. While the estimates indicate that the mites may be able to travel several meters in a short time, it is unknown whether mites sustain this dispersal rate, resulting in large distances traveled during dispersal. Also, mite movement is affected by factors such as agitation, light, temperature, and/or relative humidity, so distance achieved will likely be less than theoretical distances. Additionally, in low humidity, mites tend to clump together to avoid desiccation, which may limit their dispersal (Sánchez-Ramos et al. 2007).

Another reason 24-48 hours was chosen is that this time period is relevant to the complexity of the shelving size and distance in most retail habitats and known mite movement rates (135-227 cm/h). Applying such a barrier treatment may have limitations in preventing mites from moving among packages. However, applications should prevent mites from initiating new infestations when moving from nearby refugia sources. The shelving system in retail facilities is quite complex. There are many pathways across which mites can travel to reach shelving from the floor and to move from shelf to shelf. These pathways include support posts, shelving brackets, "backer boards," etc. Creating a barrier along these pathways can prevent mites from reaching packaging on shelves that are separated from other shelves and the floor. Further investigations should evaluate behavior and biology of *T. putrescentiae*, including movement behavior during a 24-48

hour period, sub-lethal effects of acaricides, and dispersal behavior in different humidity conditions.

Acaricides must provide efficacy at differing humidity levels on and around the retail shelving. EcoPCO DX and EcoExempt IC^2 were the only two acaricides showing mortality significantly different than the control within 24 hours and at all humidity levels. The other acaricides were affected by humidity, and, therefore, local humidity conditions should be considered when applying these other products. Within 24 hours, lower humidity levels increased efficacy of the acaricides Mother Earth D, EcoExempt D, and EcoAdjuvant, but at 68% RH, efficacy was not significantly different from the control. EcoPCO WPX, Tempo Ultra WP, and Talstar P were not significant from the control at 24 hours regardless of humidity. It has been documented that diatomaceous earth formulations have higher efficacy at lower relative humidity levels, due to their desiccant properties (Cook and Armitage 2000) and the reduced acaricide efficacy at higher humidity levels is consistent with previous documentation (Cook and Armitage 2000, Collins and Cook 2006a, Collins and Cook 2006b).

Within 48 h, all acaricides except Tempo Ultra WP and Talstar P were effective at 64 and 68% RH. The low humidity treatments (40% RH) resulted in high mortality, even within the control. This high mortality is consistent with the results of Eaton and Kells (2009). Except for the synthetic pyrethroids, all other products were effective against the mold mites at high and/or medium humidity levels.

Wet applications of the dusts, including adjuvant, were used in the first experiment to provide a uniform coating on nonporous surfaces, as earlier applications

without adjuvant resulted in acaricides beading on the shelf surface. Dusts must be applied uniformly and on the surface to provide an effective barrier. However, dusts tend to be highly visible and there are challenges with creating a uniform application with minimal visibility, hence the attempt to apply the dusts in water and adjuvant. While the adjuvant was important for spreading the dusts across nonporous surfaces, there was a question as to whether there was a synergistic or an agonistic effect of mixing the dusts in water and adjuvant. Therefore, a second experiment was needed to determine the effect of adjuvant and application method on the efficacy of the dust acaricides.

The second experiment involved three types of applications of each of the 3 dust acaricides: Mother Earth D, EcoExempt D, and EcoPCO DX. The applications were: adjuvant and dust mixed into an aqueous solution; adjuvant applied and dried, followed by a dry dust application; and dusts as a dry application only (Fig. 3). Overall, mortality was higher at 64% relative humidity than at 68% relative humidity, although this was not statistically significant. Dry applications were more effective than wet applications, showing similar results to Collins and Cook (2006a, 2006b), who tested dry and slurry applications of diatomaceous earth formulations. The results of part two of the present study indicate an agonistic effect of wet plus adjuvant applications.

With the aqueous applications, the dust seemed to adhere to the substrate, so the mites were less likely to accumulate this dust on their legs or setae, thus resulting in the reduction of efficacy. We observed accumulations of dust on mites in the dry dust treatments. Fewer dust particles on mites were observed in the aqueous treatments. Nevertheless, wet applications were important for providing a uniform coating and

reducing the overall visibility of the application. Further research on the microscopic accumulations of dust particles on mites would determine whether the differences in dust accumulation are significant. The problem of availability of the dust to the mite illustrates the difficulty in achieving an effective and discreet acaricidal barrier on the nonporous surfaces in the retail habitat.

Many studies have demonstrated various acaricidal modes of action for plant-based compounds, essential oils, and formulated acaricides, including contact toxicity, fumigant toxicity, or food additive toxicity. In the present, we study assessed the residual activity of acaricides, which is more practical for long-term control and prevention in retail facilities, as there are restrictions and limitations to the use of fumigants in retail facilities, contact applications do not provide long-term control, and there are restrictions to what mite control products could be added to a finished product.

Diatomaceous earth formulations are commonly used to control grain and stored product mites (Collins and Cook 2006a, Collins and Cook 2006b, Palyvos et al. 2006). Collins and Cook (2006a, 2006b) compared the efficacy against stored product insects and mites of dry and slurry applications of diatomaceous earth formulations on porous and nonporous surfaces. Collins and Cook (2006a) used a gloved fingertip to spread the slurried diatomaceous earth applications on the nonporous surfaces. Such applications are not practical on the large scale of the grocery store shelving. The addition of an adjuvant provided a formulation that could be applied and would spread evenly across nonporous grocery store shelving and floors. Dusts and adjuvant added to water formulations provided a uniform coating on nonporous surfaces after being sprayed to the

point of runoff.

Many plant-based and synthetic compounds have acaricidal properties, and many of these compounds have been tested alone and as part of a formulated acaricide. Benzyl benzoate is a compound commonly used as an acaricide (Harju et al. 2004, Jeong et al. 2008, Kim et al. 2003). Various essential oils and their derived compounds have been tested against the mold mite to provide alternatives to synthetic chemicals. Typically, the applications of these essential oils, derived compounds, and other plant-based compounds were studied in fumigant toxicity studies or contact exposure via an impregnated fabric disc (Kim et al. 2003, Jeong et al. 2008, Tak et al. 2006). EcoExempt D, an acaricide tested in the present study, was formulated with eugenol, a compound that showed efficacy as a contact toxin and vapor phase toxin against the mold mite in previous studies (Kim et al. 2003). EcoPCO WPX is formulated with thyme oil, which showed vapor phase and contact toxicity in previous studies (Jeong et al. 2008). Using a vapor phase or contact toxicity mode of action usually does not provide long-term control, and the acaricides may need to be reapplied periodically. The present study indicates that these plant-based compounds can be effective when used in formulated residual acaricides against the mold mite. Residual acaricides remain on the surface for a long period of time after application and, thus, allow long-term control. Additionally, residual acaricides can be applied in a way that creates a barrier between one area of the shelving to another, which creates a more targeted pest management plan.

EcoExempt IC^2, which contains rosemary oil and peppermint oil as active ingredients and requires the use of an adjuvant for mixing, demonstrated high efficacy

against the mold mite, regardless of humidity. However, this formulation is less compatible with use on retail shelving, because it left an oily residue. Perhaps it may be appropriate for use in other locations, such as crack-and-crevice treatments or spot applications away from shelving. Further research in additional situations would determine the appropriateness of these applications of EcoExempt IC^2. In addition to potential usefulness in refugia, EcoExempt IC^2 may provide efficacy against mites crossing a small treated area. These potential applications should be tested for efficacy against *T. putrescentiae* and other stored product mites.

In addition to contact or fumigant toxicity, plant-derived compounds, essential oils, or crushed plant materials can be mixed within a food commodity to manage mold mite infestations (Hubert et al. 2008, Gulati and Mathur 1995). In addition to using essential oils and plant-based compounds as food additives, formulated acaricides have also been tested in different concentrations in mold mite diets (Hubert et al. 2007). While the previously mentioned studies provide useful information about the acaricidal activity of plant-based extracts and compounds, a fumigant mode of action is not suitable for an acaricide used in retail, contact acaricides may be too short-acting to prevent mites from spreading in retail facilities, and food additives may affect palatability of the food product. Nonetheless, theses studies offer insight as to which compounds may be useful in formulating acaricides for use against the mold mite.

Efficacy studies of formulated acaricides have included acaricides with the following active ingredients: abamectin, benzyl benzoate, organophosphates, synthetic pyrethroids, fipronil, azadiracthin, insect growth regulators, pyriproxyfen, and spinosad

(Stará et al. 2011, Hubert et al. 2007). Acaricides were tested using different applications, including contact, vapor phase, or food-additive (Stará et al. 2011, Hubert et al. 2007). Other than diatomaceous earth, none of the above acaricides (mainly the synthetic pyrethroids) were tested against mold mite using a residual application until the present study.

The present study demonstrated the efficacy of dusts and essential oils applied as formulated and registered residual acaricides against the mold mite in simulated retail surfaces. There are limitations or restrictions on current labels for applying acaricides around finished food products. These limitations can make it difficult to select an effective acaricide for use in the retail setting, so the products tested were compatible with use in the retail setting. Future research should involve evaluating the efficacy of these products in the retail habitat. Additionally, an effective integrated pest management (IPM) program should be considered as there are areas where products even with an inclusive label would be prohibited from use. Considering that store humidity is variable on and under shelving and humidity affects acaricide efficacy, humidity should be monitored in order to assess the impact on mite control methods. Sanitation and the reduction of humidity should be used in combination with acaricides to help prevent the spread or dispersal of infestations of mold mites.

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Chapter 3. Explosive dispersal behavior of the mold mite, *Tyrophagus putrescentiae* (Acari: Acaridae): the influence of relative humidity

Summary

The mold mite, *Tyrophagus putrescentiae* (Schrank), is a stored product pest that can damage many types of food. Infestations may remain undetected in food sources for long periods, where populations may increase and food sources become depleted, resulting in dispersal of mites to new habitats that have suitable nutrition and humidity. The objective of this study was to better characterize dispersal characteristics of this mite, in relation to changing ambient humidity. Arenas were designed with an enclosed food reservoir filled with ground dog food and incubated at high humidity. The remainder of the arena was incubated at one of three humidity levels (69.5, 41.6, or 32.0% RH) to provide a space for mites leaving the food reservoir. The number of mites leaving the food reservoir was monitored daily until the dispersal mode was determined, and final population characteristics were analyzed. In this experiment, mites exhibited "explosive dispersal," when a migration occurred *en masse,* suddenly within 24 h, and was dependent on increasing mite density. Dispersal was significantly affected by relative humidity, as humidity affected the mite densities in the food. *In situ*, large numbers of mites may be present in food packages and may go undetected until the package is opened or explosive dispersal has occurred. Direct sampling, instead of trap monitoring, may be necessary to detect infestations before explosive dispersal has occurred. If baited traps are used to monitor infestations, there is a risk of false negatives occurring, i.e. actual presence of mite infestations when no mites have been detected in nearby traps.

Introduction

The mold mite, *Tyrophagus putrescentiae* (Schrank), is a stored product pest that can cause substantial economic damage. This mite utilizes a diversity of foods such as grains (Hughes 1976), cheese in cheese houses (Robertson 1952), cured ham (Arnau & Guerrero 1994) and pet food stored in warehouses as well as retail outlets (Brazis et al. 2008). In addition to the economic impact of destroying food, there is evidence that the mite may be a source of allergens affecting dogs and humans (Mueller et al. 2005).

Mite infestations may remain hidden and undetected for long periods. As the population increases and food sources become depleted, mites will disperse to new patches that have suitable nutrition and humidity. Directionality of intra-patch movement is likely a result of food volatiles and humidity (Skelton et al. 2007). However, evidence for dispersal is often after-the-fact, depending on the attentiveness of workers to detect the accumulation of mites and biomass on the food shelves. Mite accumulation may be limited to small clumps or aggregations of mites on food shelves. However, most infestations will not be detected until personnel find that shelves and packages have become extensively covered in mites and have a dusty or neglected appearance. The cryptic nature of this mite, coupled with the sudden onset of mite outbreaks has therefore made mite management quite challenging.

There have been few studies on *T. putrescentiae* dispersal in structures, including an analysis of triggers and mechanisms of dispersal. Mold mites can infest new dog food packages via active ambulatory dispersal (i.e. walking from one food package to another) and this dispersal may be triggered by external or internal environmental conditions or

cues. The alarm pheromone, neryl formate, is likely involved, but little is known about the resultant mite behavior in response to this compound (Kuwahara et al. 1989), or to environmental triggers in general.

With other mite species, there are a number of external and internal environmental conditions that trigger dispersal. For example, *Tetranychus urticae* Koch movement was found to be affected by habitat, food source, humidity levels, and light levels (Hussey and Parr 1963). Specifically, humidity hindered movement, mites were positively phototactic (moved toward light), and mites moved toward the center of the landscape of food sources (Hussey and Parr 1963). In predatory mites of the genus *Neoseiulus* (Acari: Phytoseiidae), dispersal is often affected by environmental conditions and food availability (Auger et al. 1999, Coop and Croft 1995). Other factors affecting dispersal of these mites include wind and temperature (Coop and Croft 1995). Factors affecting dispersal of *Neoseiulus californicus* (McGregor) include temperature, prey availability (predator-to-prey ratio), light intensity, relative humidity (RH), and condition of foliage (turgid versus withered) (Auger et al. 1999). Dispersal was reduced at lower temperatures (15°C) and increased at medium and high temperatures (25-35°C) (Auger et al. 1999). As prey density decreased, dispersal increased, as the mites searched for new food sources or risked starvation (Auger et al. 1999). *Neoseiulus californicus* is negatively phototactic, so higher light intensities resulted in higher ambulatory dispersal (Auger et al. 1999). In order to prevent water loss/desiccation, *N. californicus* mites tended to disperse more when RH was low (55%) versus high (80%) (Auger et al. 1999). Additionally, phytoseiid mite eggs are sensitive to dehydration, so dispersal also may

have been to find a more suitable place to lay eggs (Auger et al. 1999). Mites placed on drought-stressed/wilted alfalfa were also more likely to disperse than those placed on turgid alfalfa (Auger et al. 1999). This could also be due to microclimatic effects, such that the area around drought-stressed alfalfa has a lower RH and higher temperature, both of which tend to increase dispersal from the site (Auger et al. 1999). Previous research has shown numerous triggers and mechanisms of mite dispersal, but did not analyze population characteristics and timing with regard to dispersal. It may be important to examine dispersal from a population scale, instead of analyzing the dispersal patterns of a small number of mites.

In addition to the stimuli that cause dispersal, there is also the response of the population to these stimuli. Different types of active dispersal occur in the animal kingdom; while some of these dispersal strategies are continuous—with an unvarying portion of the population dispersing each generation—sometimes a non-continuous strategy may be representative of a species dispersal strategy (Bowler and Benton 2005). Explosive dispersal is the mass movement of a group of organisms from their habitat over a short period of time (Aylmer and Reisner 1971). Continuous dispersal is the gradual movement of organisms over time from the favorable habitat. Explosive dispersal is exemplified in paramecium, migratory locusts, and microtone rodents (Rodentia: Cricetidea, Arvicolinae) (Aylmer and Reisner 1971, Ekerholm et al. 2001, Lovejoy et al. 2006). Examples of continuous dispersal include movements of bison, Mammalia: *Bison bison* (L.), and German cockroaches, *Blattella germanica* (L.) (Larter et al. 2000, Runstrom and Bennett 1984). Knowing the dispersal characteristics is important for

understanding how mites exploit patches within a structure, as well as development of effective appropriate IPM practices. When attempting to detect a pest such as *T. putrescentiae*, continuous dispersal would benefit monitoring programs where traps would begin to pick up mites early in the infestation. Knowing that a pest undergoes explosive dispersal would require additional monitoring tactics to be developed.

Before considering mechanisms of dispersal we must determine the type of dispersal exhibited by *T. putrescentiae* and possible environmental conditions that affect this dispersal. As this mite is desiccation-intolerant, humidity may be a factor mediating dispersal. Population density of mites may also affect dispersal of mold mites. These characteristics can be examined by analyzing the patterns of mite population development and dispersal at different humidities, the time interval prior to dispersal, and the population characteristics at time of dispersal. Knowing these characteristics will help understand dispersal potential with relation to environmental conditions, such as RH, and timing of dispersal for further studies.

Materials and Methods

Mold mites, *Tyrophagus putrescentiae* (Schrank), were reared in 0.946 L Mason® jars on semi-moist dog food as per Eaton and Kells (2011). Jar lids were replaced with mesh screen with filter paper (P5, Fisher Scientific, Pittsburgh, PA) on both sides of the screen to provide ventilation and reduce mite escape. These modified lids were held on by securing the band on the screw threads. The jars were placed in larger plastic containers with water to maintain high humidity. Jars were incubated at room

temperature (22-25°C). The lids of the larger containers had 4 holes (1.3 cm dia.) for ventilation.

Eighteen arenas were constructed using cast acrylic (10 cm by 10 cm and 0.5 cm thick, McMaster-Carr, Chicago, IL) sheeting as floor and ceiling and high density polyethylene (HDPE, 10 cm by 10 cm and 0.47 cm thick, McMaster-Carr, Chicago, IL) sheeting with a 7 cm dia. hole cut into the center to act as wall for each arena (Fig.1). Five holes were cut into the cast acrylic sheet that acted as the floor for each arena—one hole in the center and four holes around it. The center hole was used as a food reservoir. A piece of filter paper (P5, Fisher Scientific, Pittsburgh, PA) was glued underneath the hole to prevent mite escape, and a piece of mesh (Precision Woven Nylon Mesh, 193 X 193 Mesh, 0.079 mm) was glued (Arrow all purpose clear glue sticks, Saddlebrook, NJ) over the filter paper to support this paper. Mesh was glued over top of the outer four ventilation holes. Holes (1.25 cm) were punched in the lid of 14 mL round-bottom culture tubes (Falcon Brand, BD Biosciences, Franklin Lakes, NJ, USA), and the lids were glued under the five ventilation holes of the arena (including the food reservoir). These lids held culture tubes with saturated salt solutions to maintain the prescribed relative humidity (RH). In setting RH, four tubes containing an appropriate salt solution were affixed to the four holes surrounding the central hole, which held the food reservoir. The food reservoir was incubated at high humidity in all arenas.

Three different saturated salt solutions were used to maintain a set humidity. Potassium nitrate (KNO_3) was used under all food reservoirs to provide a high humidity

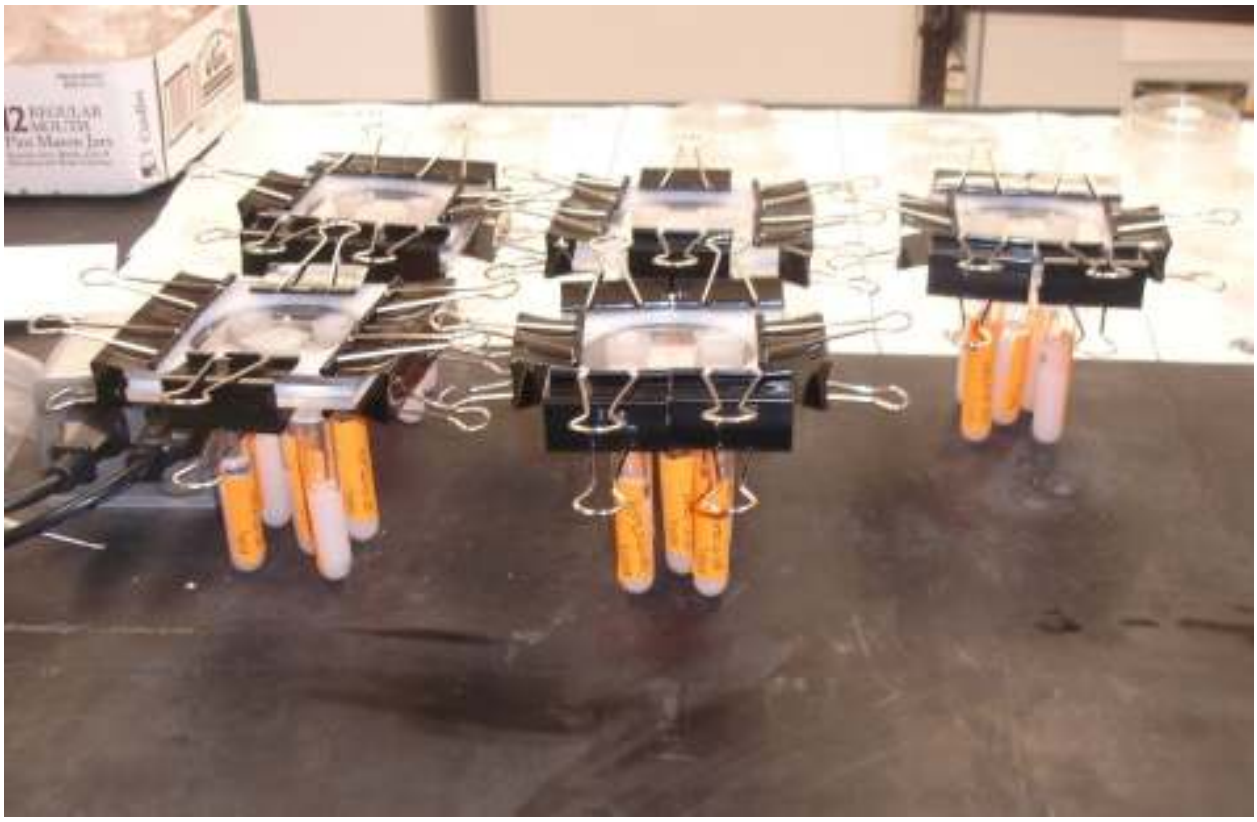

Figure 1. Photograph of assembled humidity arenas. Arenas consisted of two clear cast acrylic plates with a high-density polyethylene (HDPE) plastic layer in between. A hole in the HDPE layer provided the arena walls. Tubes underneath the arena provided a means to adjust humidity using saturated salt solutions.

for the food. Three salt solutions under the vent holes were used to set the desired RH in each arena: potassium nitrate (85-97.5% RH), potassium carbonate (40-47% RH), and lithium chloride (11-14.5% RH). Humidity in the arena was measured by using a plastic arena blank with the same dimensions as the HDPE layer, but having a hole that enabled direct measurement of humidity (via a Vaisala RH meter, model HMI41 with a HMP42 probe; Helsinki, Finland). Actual humidity measured in the arenas at room temperature (23 – 25˚C) were 69.5, 41.6 and 32.0% RH for high, medium, and low RH, respectively.

Ground dog food (150 mg ± 0.238 mg) was placed into each central food reservoir. Twenty (20) adult mites were placed in the food reservoir and the hole was covered with Parafilm (Bemis Flexible Packaging, Neenah, WI) to maintain high humidity. A small hole was made with a #2 insect pin (BioQuip, Rancho Dominguez, CA) in the Parafilm to allow the mites to move through the barrier. Arenas were secured via six (6) large binder clips (clip size 5.08 cm, capacity 2.699 cm, ACCO, Lincolnshire, IL) to prevent escape of the mites.

The number of mites leaving the food reservoir was monitored every 24 hours for 85 days or until dispersal mode (continuous or explosive) was determined. At the end of the trial time or when explosive dispersal was observed, the arenas were frozen to stop development, and the population characteristics were analyzed and recorded. The average number of days prior to dispersal was recorded and means (standard errors) were calculated within each humidity using Proc Means in SAS Statistical Software (SAS Institute 2011).

Mites by stage of growth were enumerated separately within the arena area and the food reservoir. Due to the large number of mites in the food, representative samples were taken from the dispersed food and from the food reservoirs to estimate mite abundance. The total mass of the food reservoir contents was measured in a tared centrifuge tube. Next, five to ten samples of approximately 0.5 mg were removed and the number and stages of mites were enumerated for each sample. Using SAS Statistical software, final population characteristics data were log transformed and analyzed using Proc GLM, and mite stage and arena humidity were used as variables (SAS Institute 2011). Differences were tested through adjusted (Least Squares) means, with results reported as median and 95% confidence interval (CI) of the back-transformed means.

Results

The type of dispersal observed in the arenas was characteristic of explosive dispersal. At high relative humidity (69.5%), all 6 arenas exhibited this type of dispersal, and the average time to explosive dispersal was 32 ± 3 days (Fig. 2). At the moderate relative humidity (41.6%), 4 of the 6 arenas exhibited dispersal, and the remaining two arenas failed to show any dispersal. When explosive dispersal occurred, the average dispersal time at 41.6% RH was 56 ± 4 days (Fig. 2). At low relative humidity (32.0%), none of the 6 arenas exhibited dispersal, and excessive mortality was observed, even in the food reservoirs (Fig. 2).

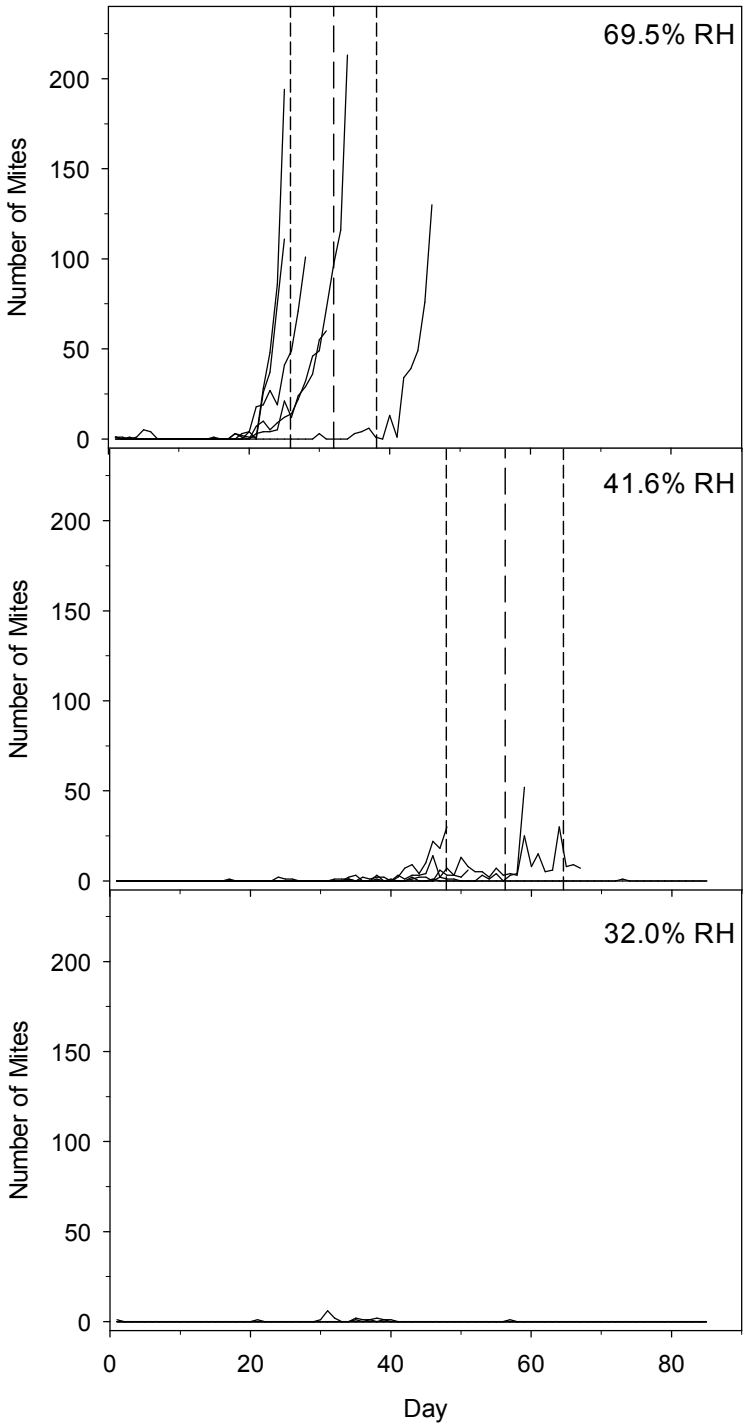

Figure 2. Mean number of days to explosive dispersal as affected by relative humidity. Mean days to explosive dispersal is denoted by the central vertical line (– – – –) bounded by the 95% CI (-----).

The age distributions at the final day varied by humidity (Table 1). At low humidity a majority (84.3%) of the mites were adults. At high humidity, the age distribution for the total of the arena, including the reservoir and dispersed areas of the arena, was 13.8% adult, 44.4% immature, and 41.8 % egg. At medium humidity, the age distribution for the total number of mites in each arena was 41.1% adult, 42.2% immature, and 16.7% egg. For arenas at medium humidity that only exhibited explosive dispersal, the age distribution was 34.1% adult, 51.3% immature, and 14.6% egg.

Final population densities varied by humidity (Table 2). In arenas where explosive dispersal occurred, the density at time of explosive dispersal was calculated. Total density of mobile (immature + adults) mites included those mites remaining in the reservoir and those dispersing from this reservoir. The median total density per mg of food was 74.7 (95% CI: 49.7, 112.4) at high humidity and 6.1 (95% CI: 0.446, 85.1) at medium humidity (Table 3). In the food reservoirs only, the median density of mites per mg of food was 63.4 (95% CI: 34.0, 118.4) at high humidity and 5.6 (95% CI: 0.35, 85.6) at medium humidity.

Dispersed mites in the rest of the arena were simply counted. At high humidity, median number of mites found outside the food reservoir were 99.5 (95% CI: 46.2, 212.7) adult mites, 319.5 (95% CI: 121.3, 839.0) immature mites, and 125.5 eggs (95% CI: 64.2, 244.5) (Fig. 3). As eggs cannot disperse actively on their own, eggs found outside of the food reservoir were results of ambulatory mites: either laid by dispersing females, moved via Brownian motion with dispersing mites, or deposited after being

Table 1. Age distribution at final day of study, or after an explosive dispersal event.

		Percent of Population		
Stage	Humidity	*Arena* [1]	*Reservoir*	*Total*
Egg	High	23.0	45.1	41.8
Immature	High	58.7	41.3	44.4
Adult	High	18.3	13.7	13.8
Egg	Medium[2]	0.6	17.9	16.7
Immature	Medium	24.6	45.1	42.2
Adult	Medium	74.8	36.9	41.1
Egg	Med Disp. [3]	0.4	15.0	14.6
Immature	Med Disp.	34.8	51.8	51.3
Adult	Med Disp.	64.8	33.2	34.1
Egg	Low	0.0	8.5	3.1
Immature	Low	5.4	31.4	12.6
Adult	Low	94.6	60.2	84.3

[1]- Mites in arena were dispersed mites, while mites in reservoir did not disperse.

[2]- The total number of mites includes those that dispersed and those that did not disperse.

[3]- Med disp. includes calculations only for the arenas at medium humidity that exhibited explosive dispersal.

Table 2. Minimum and maximum counts in arena by location, stage of mite, and humidity level in the arena.

		Dispersed		Reservoir		Total[2]	
Stage	Humidity[1]	Min	Max	Min	Max	Min	Max
Adult	High	36	506	712	5169	1219	5240
Adult	Medium – ND[3]	5	90	4	6816	9	6865
Adult	Med – D[3]	49	90	499	6816	567	6865
Adult	Low	3	33	1	22	4	43
Egg	High	37	407	2725	20793	3131	20950
Egg	Medium - ND	0	1	3	6892	3	6893
Egg	Med – D	0	1	167	6892	168	6893
Egg	Low	0	0	0	11	0	11
Immature	High	98	2616	1681	19099	4297	19316
Immature	Medium - ND	0	114	3	12594	3	1261
Immature	Med – D	17	114	1485	12594	1529	12611
Immature	Low	0	10	0	25	0	32

[1]-High, Medium, and Low Humidity refer to 69.5, 41.6, and 32.0 % Relative Humidity (RH), respectively.

[2]-Total includes the number of mites dispersed plus the number of mites in reservoir.

[3]-Medium –ND includes only counts for arenas at medium humidity that did not exhibit explosive dispersal. Med – D includes only counts for the arenas at medium humidity that exhibited explosive dispersal.

Table 3. Mite density in reservoir and total mites in arena as a function of mass of food in the reservoir at time of explosive dispersal.

Relative Humidity (RH %)	Number of Mites / mg of food			
	Reservoir		Total	
	Median	95% CI	Median	95% CI
69.5	63.4	(34.0, 118.4)	74.7	(49.7, 112.4)
41.6	5.6	(0.4, 85.8)	6.1	(0.4, 85.1)

carried on the bodies of dispersing mites. At medium humidity, the number of mites found outside the food reservoir depended upon whether or not explosive dispersal occurred (Fig. 3). In the arenas held at medium humidity were explosive dispersal occurred, the median number of mites outside the food reservoir was 66.0 (95% CI: 51.6, 84.2) adults, 35.5 (95% CI: 14.5, 84.8) immature mites, and 0.4 (95% CI: -0.04, 1.1) eggs. (Fig. 3) No explosive dispersal occurred in the arenas incubated at low humidity, and the median number of dispersed adults was 8.6 (95% CI: 4.2, 16.7) (Fig. 3). The range for the number of dispersed mites was large for each developmental stage and humidity combination (Fig. 3).

In arenas exhibiting explosive dispersal, a median number of adult mites in the food reservoir was 2372.4 (95% CI: 1357.2, 4146.4) at high humidity and 2520.9 (95% CI: 508.6, 12479.9) at medium humidity (Fig. 4). Also in these food reservoirs of the arenas exhibiting explosive dispersal, the median number of immature mites was 7159.4 (95% CI: 3752.7, 13657.7) at high humidity and 3935.9 (95% CI: 1157.6, 13376.7) at medium humidity. In these arenas, the median number of eggs was 7823.7 (95% CI: 4360.1, 14038.2) at high humidity and 1135.2 (95% CI: 137.7, 9307.4) at medium humidity (Fig. 4). Low mite numbers were found in the food reservoirs of arenas that did not exhibit explosive dispersal. The total number of mites in the arena was calculated by adding dispersed mites plus mites in the food reservoir (Fig. 5).

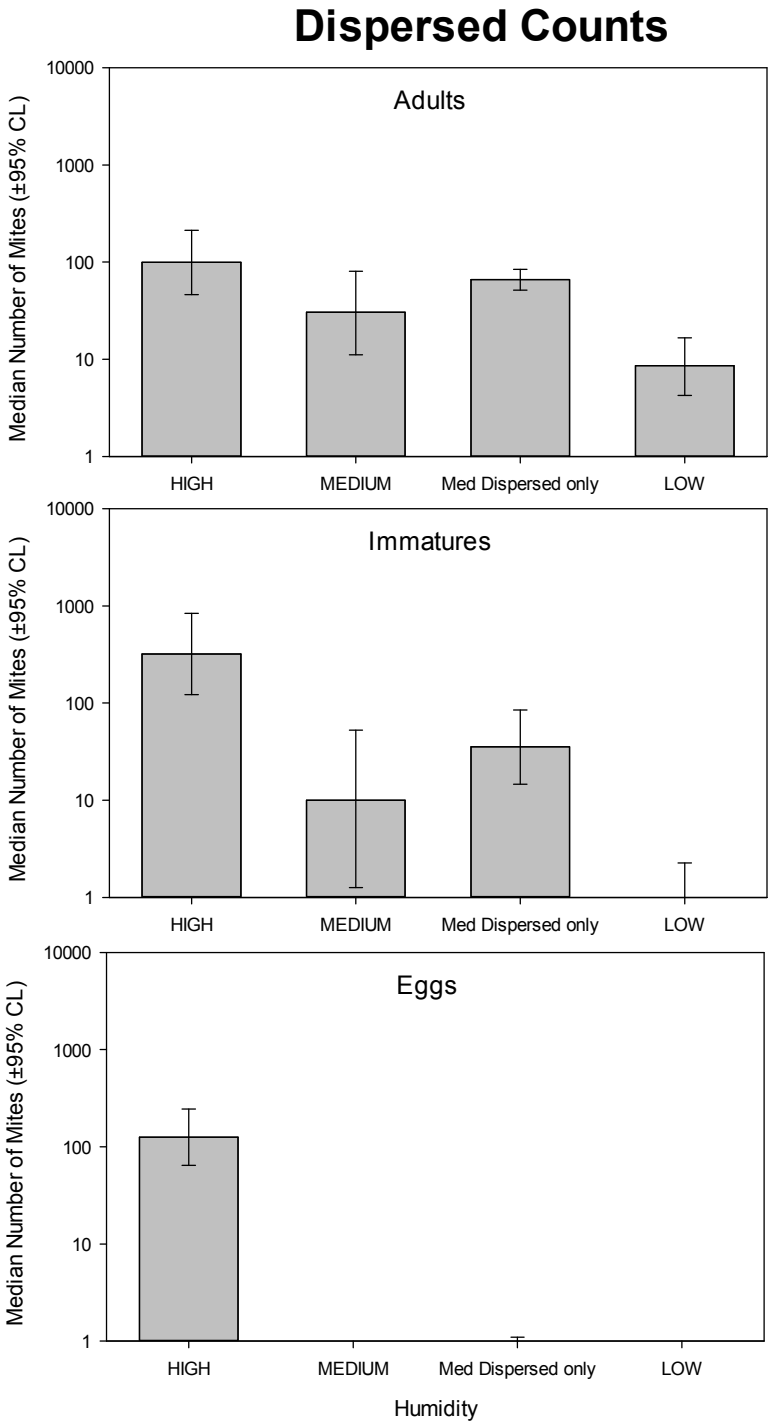

Figure 3. Final counts of dispersed mites in each arena. The data are log-transformed, resulting in median numbers asthe central estimation of mites.

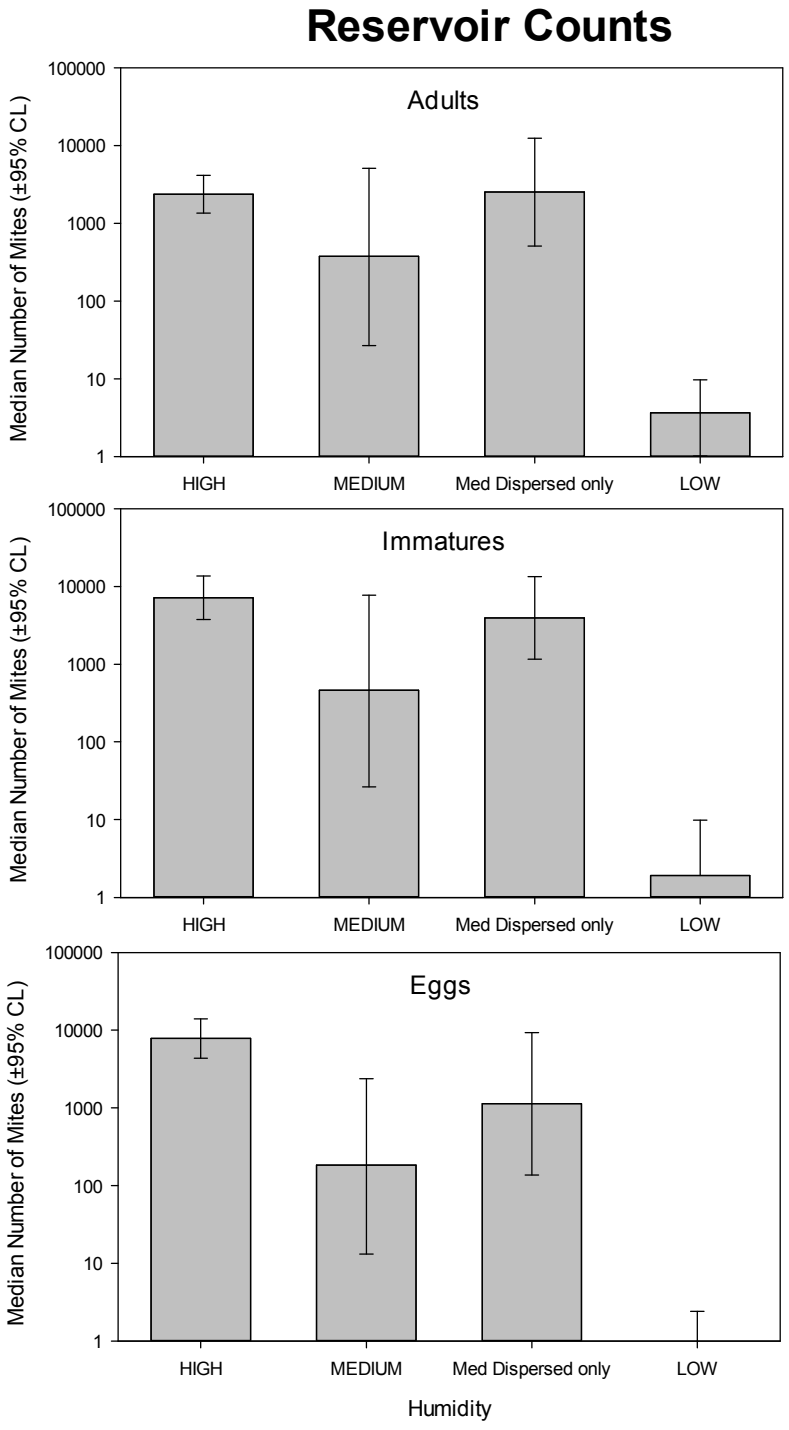

Figure 4. Final counts of mites in food reservoirs in each arena. The data are log-transformed, resulting in median numbers as the central estimation of mites.

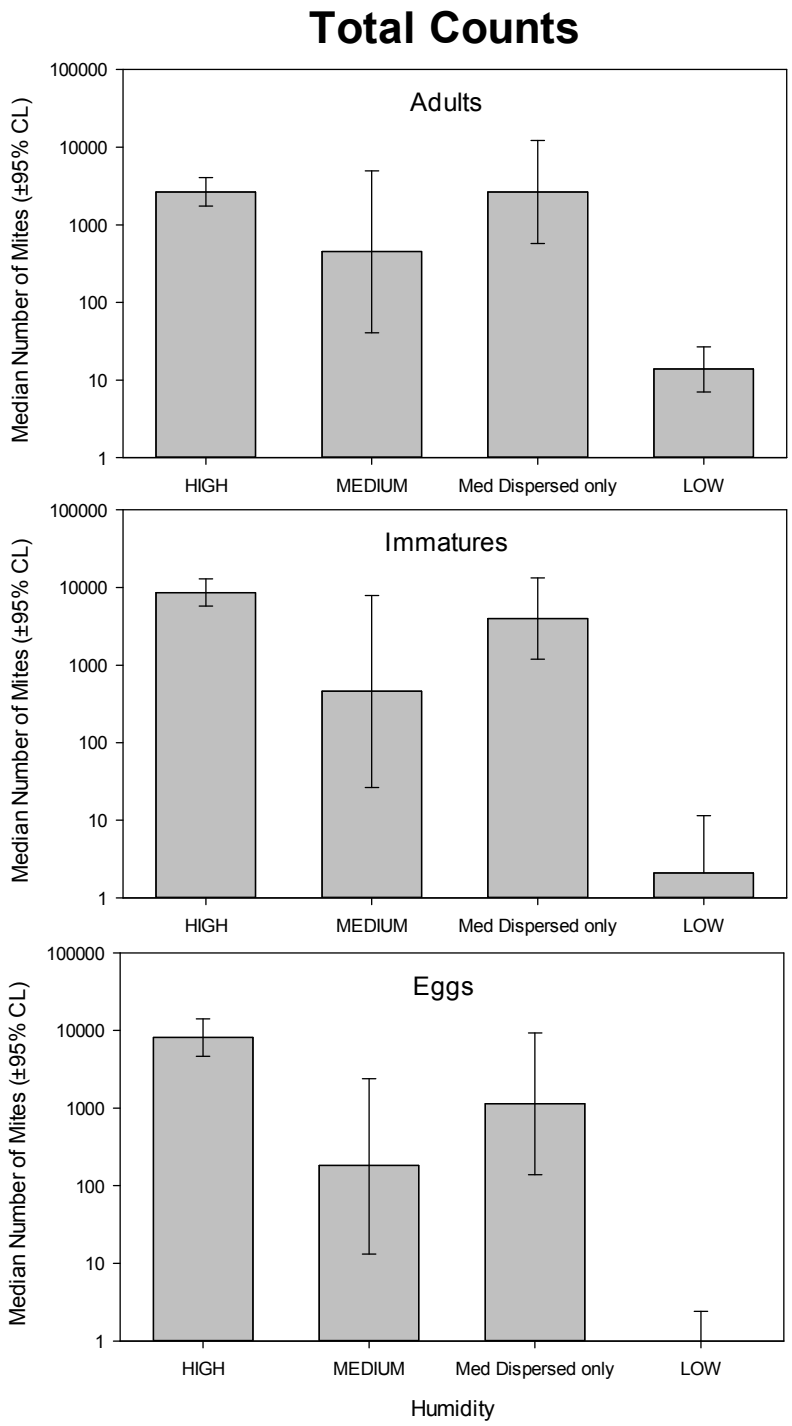

Figure 5. Final counts of all mites in the arenas, dispersed and not dispersed. The data are log-transformed, resulting in median numbers as the central estimation of mites.

The intrinsic rate of increase (r_m) was calculated using the following equation (Andrewartha and Birch 1954):

$$r_m = \log_e(N_t/N_0)/t$$

In arenas that exhibited explosive dispersal, the intrinsic rate of increase (r_m) was 0.2227 ± 0.0157 (SE) at 69.5% RH and 0.0955 ± 0.0218 (SE) at 41.6% RH. In arenas that did not exhibit explosive dispersal, the r_m was 0.0125 ± 0.0158 (SE) at medium humidity and -0.0017 ± 0.0056 (SE) at low humidity.

Discussion

The type of dispersal displayed by mites in this experiment was explosive dispersal behavior, where a migration occurred *en masse* suddenly and was dependent on increasing mite density. While explosive dispersal was expected at low humidity, conditions in the arena were severe enough to affect humidity in the food reservoir and affect population development so dispersal was not achieved. However, explosive dispersal was previously encountered at low humidity when a bag of infested pet food was stored at low humidity (~10% RH) in a laboratory office. A larger mass of food in the package and barriers to control moisture loss enabled mites to build a sufficient density. The resulting dispersal event resulted in mites moving up to 3 m from the site of infestation within 48 h. This observation was the impetus for evaluating dispersal behavior over a range of humidities. High humidity arenas were expected to have mite numbers and debris accumulation gradually occur over time in arenas, as this humidity was favorable for mite survival and development. However, the mites tended to stay within the food patch. There was intermittent movement of mites at low numbers in all arenas, but it was expected that mites would use the arena with increasing frequency as their population numbers increased. However, instead of the slow accumulation of mites active in the arena, mites suddenly moved *en masse* during the 24 h between observations, regardless of humidity.

In arenas where explosive dispersal occurred, there was more reproduction and population growth than in those arenas with no dispersal. Dispersal was affected by relative humidity in that it affected the mite densities in the source food. Our intrinsic

rate of increase (r_m) values for arenas that reached explosive dispersal were 0.2227 ± 0.0157 at 69.5% RH and 0.0955 ± 0.0218 at 41.6% RH. Literature values for r_m depend on temperature and RH. At 20°C and 90% RH, r_m was calculated at 0.2052 ± 0.0017, while at 25°C and 90% RH r_m was 0.3189 ± 0.0038 (Sánchez-Ramos and Castañera 2005). Other studies examined the population dynamics of *T. putrescentiae* at 25°C and different relative humidities and calculated an r_m of 0.1645 ± 0.0020 at 80% RH, 0.19 ± 0.01 at 85%, 0.3173 ± 0.0040 at 90% RH (Sánchez-Ramos et al. 2007, Aspaly et al. 2007). Our calculation for r_m at room temperature (22-25°C) and high relative humidity in the arena (69.5%) is within the range of those found in the literature. While the arena itself was at 69.5% RH, it should be noted that the RH of the food was likely higher, as the headspace of this solution had a measured RH of 82.0%. This high reproductive potential of the mold mite results in a rapid increase in infestations if environmental conditions are favorable and a food source is present. At higher humidities, mite numbers reached 74.7 (95% CI: 49.7, 112.4) mites/mg at 69.5% RH and 6.1 (95% CI0.4, 85.8) mites/mg at 41.6% RH at the point of explosive dispersal.

Relative to the point where dispersal was determined, the age distribution was also estimated. The stable age distribution is 56% eggs, 34% immature, and 9.7% adults, for mites reared on brewer's yeast at 13-15°C (Barker 1967). The present study took place at room temperature (22-25°C), which is higher than in Barker's experiment (1967). The age distribution varied with humidity and included a higher percentage of adults, with an overall age distribution of 84.3% adults, 12.6% immature, and 3.1% eggs. At medium humidity, the age distribution was 41.1% adult, 42.2% immature, and 16.7%

egg, while in the medium humidity arenas exhibiting explosive dispersal, the age distribution was 34.1% adult, 51.3% immature, and 14.6% egg, and in the medium humidity arenas without dispersal, the age distribution was 46.8% adult, 37.1% immature, and 16.0% egg. At high humidity, the age distribution approached the stable age distribution at 13.8% adult, 44.4% immature, and 41.8 % egg. As the stable age distribution is reached, there is a chance that explosive dispersal may occur, which could affect the outcome of experiments, particularly when behavior is involved.

Habitat space can be heterogeneous, and patches of habitat may be interspersed within patches of non-habitat, often referred to as matrix (Hanski & Ovaskainen 2003). This is certainly true for *T. putrescentiae*, where habitat could include food products on retail shelves, food dust, and insect debris in cracks and crevices, food accumulation in processing equipment, stored grains, other insect infestations, soil, leaf litter, plants, fungus, cheese, and cured meats (Hughes 1976, Brust and House 1988, Papadopoulou 2006, Kheradmand et al. 2007, Duek et al. 2001, Smrž and Čatská 1987, Fan and Zhang 2007, Robertson 1952, Arnau & Guerrero 1994). Although separated by matrix, patches in fragmented habitats can be connected in two basic ways: structurally or functionally (Kindlmann & Burel 2008). Structural connectivity is where there is some form of physical pathway in the landscape structure that connects the patches, whereas functional connectivity takes into account the behavior and adaptations of the focal species in response to the landscape (Kindlmann & Burel 2008). Functionally, to successfully move among patches, mites move *en masse*, a strategy that may improve chances of

locating new patches or increase the probability of successful colonizers. High humidity might improve distance of dispersal as mites transverse to access new patches.

In identifying triggers of dispersal, it has been shown that dispersal potential [distance and rate of movement] for mites may vary greatly, depending on species of mite (Ojala and Huhta 2001). In the case of *T. putrescentiae*, understanding conditions forcing dispersal, leads to opportunities to investigate more specific dispersal triggers for mold mites and the potential for infestation spread. Learning more about the dispersal behavior and population dynamics of *T. putrescentiae* will provide insight into ways to prevent and control this pest. At present, that this mite explosively disperses presents a problem to practitioners when attempting to monitor and detect infestations of this mite in retail facilities. The tendency for mites to build up within the food source to very high numbers makes it difficult to detect an infestation before mass movement occurs. The densities found in this study translate to a potential range of 61,000,000 to 750,000,000 mites per 10 kg bag of dog food. The potential for these large numbers of mites in dog food packages is troubling, particularly because they may not be detectable until the package is opened or explosive dispersal has occurred from the infested package. At the very least, direct sampling of habitat is necessary to detect infestations and the degree of infestation. If baited traps are used to monitor for infestation, practitioners must realize there is a risk of false negatives occurring, i.e. presence of a mite infestation when no mites have occurred in the trap.

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