MONITORING POPULATIONS OF THE HAM MITE, *TYROPHAGUS PUTRESCENTIAE* (SCHRANK) (ACARI: ACARIDAE): RESEARCH ON TRAPS, ORIENTATION BEHAVIOR, AND SAMPLING TECHNIQUES

by

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AN ABSTRACT OF A DISSERTATION

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Abstract

The phase-out of methyl bromide production, the most effective fumigant for the control of the ham mite, *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae), on dry-cured ham has necessitated the search for other management methods. The foundation of a successful management program is an effective monitoring program that provides information on pest presence and abundance over time and space to help in making management decisions. By using the standard trap made from disposable Petri dishes and a dog food-based bait, mite activity was monitored weekly in five dry-cured ham aging rooms from three commercial processing facilities from June 2012 to September 2013. Results indicated that mite numbers in traps in facilities typically had a pattern of sharp decline after fumigation, followed by a steady increase until the next fumigation. Average trap captures varied due to trap location, indicating that traps could be used to identify locations where mite infestation of hams may be more likely to occur. Experiments were also conducted in 6 m x 3 m climate-controlled rooms to determine the effects of some physical factors on trap capture. Factors such as trap design, trap location, trap distance, duration of trapping, and light conditions had significant effects on mite capture. Mites also responded differently to light emitting diodes of different wavelengths, either as a component of the standard trap or as a stand-alone stimulus to orientation. To determine the relationship between trap capture and mite density, experiments were carried out in the climate-controlled rooms. Mite density was varied but trap number remained constant for all mite densities. There was strong positive correlation between trap capture and mite density. In simulated ham aging rooms, the distribution of mites on hams was determined and different sampling techniques such as vacuum sampling, trapping, rack sampling, ham sampling and absolute mite counts from whole hams were compared and correlated. Results showed weak or moderate correlations

between sampling techniques in pairwise comparisons. Two sampling plans were developed to determine the number of samples required to estimate mite density on ham with respect to fixed precision levels or to an action threshold for making pest management decisions. Findings reported here can help in the optimization of trapping and sampling of ham mite populations to help in the development of efficient, cost-effective tools for pest management decisions incorporated with alternatives to methyl bromide.

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I dedicate this work to my husband, Kumanyini Gyamfi, and our son, Brempong Gyamfi, without whose sacrifice, love, support, and encouragement, I would not be where I am today. Thank you for being with me every step of the way and making this dream a reality! YES, WE DID IT!

With love,

Yaa Amoh.

Chapter 1 - Literature Review, Relevance of Study and Objectives

Literature Review

The methyl bromide problem

The dry-cured ham industry in the United States is faced with the ham mite, *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) pest problem that may not have been of great concern until the ban of methyl bromide. Methyl bromide is an odorless, colorless gas produced industrially and biologically from naturally occurring bromide salts and had been used as a pesticide across a wide range of agricultural sectors (USDA 2000, Fields and White 2002, EPA 2006). Methyl bromide has been the most effective fumigant for the control of the ham mite (Rentfrow et al. 2006), however, it depletes the ozone layer. It has therefore been banned as a result of the Montreal Protocol, an international treaty developed in 1987 to protect the ozone layer by phasing out ozone depleting substances. The Clean Air Act, a United States federal law, originally passed in 1973 and amended in 1990, defines the U.S. Environmental Protection Agency's responsibilities for protecting and improving air quality and the ozone layer to help protect human health and the environment (EPA 2008). Under the Montreal Protocol and the Clean Air Act, the amount of methyl bromide produced and imported in the U.S. was therefore reduced incrementally until the pesticide was phased out in 2005 (EPA 2006).

Since there had been no feasible alternative to methyl bromide for the control of the ham mite, methyl bromide has continued to be available under critical use exemptions which are permitted under the Clean Air Act and the Montreal Protocol. However, methyl bromide critical use nomination for post-harvest use on dry-cured pork products submitted in 2015 for use in 2017 was withdrawn on the basis that sufficient amount of pre-phase-out stocks are available to meet the amount of 3240 kg methyl bromide that was requested. As of December 2014, EPA’s annual inventory report stated that there was still about 158 metric tons of methyl bromide

available as pre-phase-out stock. Ham producers will therefore need to rely on the existing stock after which other alternatives must be used (EPA 2017).

This study was a part of a broader objective to establish effective alternatives to methyl bromide fumigation for the control of the ham mite. Many ham processing facilities rely heavily on methyl bromide to control the pest and have been affected by the limited availability of methyl bromide since they have mite infestations every year and would fumigate whenever they see a ham mite (Rentfrow et al. 2006). The development and implementation of an effective integrated pest management (IPM) program for the dry-cured ham industry is therefore important. Interestingly, up to this time there has been no direct work toward modern IPM for the ham mite in context of the ham industry.

Biology of *T. putrescentiae*

Tyrophagus putrescentiae, commonly referred to as the mold mite, the cheese mite, or the ham mite, is about 0.2 - 0.5 mm long with a translucent body and colorless chelicerae and limbs (Hughes 1961). The life cycle consists of the egg, larva, protonymph, tritonymph and adult. Adults often mate repeatedly with one male capable of fertilizing as many as 450 females. The source of food significantly affects the reproductive rates of females. When reared on wheat germ or yeast, a female mating twice a month can produce approximately 500 eggs, with up to 60 eggs/ day, with an average of 4 eggs/day. About 70% of these are laid during the first three weeks of adult life (Boczek 1991). Oviposition can occur above 8 °C, with optimal oviposition occurring between 22 - 26 °C. Fecundity is dependent on the number of males per female as females lay fewer eggs if mated by numerous males and this can be reduced by up to 50% when male: female ratio is 4:1. Females predominate and sex ratio is not dependent on food, parental

age or frequency of copulation (Boczek 1975). The development from egg to adult usually takes about one to three weeks and could take up to 118 days depending on the environmental conditions and the type of food on which the mite is reared (Kheradmand et al. 2007). No development has been reported at temperatures below 8.5 °C or above 36 °C (Cunnington 1969). *T. putrescentiae* males live longer than females, and longevity reduces as temperature increases (Sánchez-Ramos and Castañera 2005).

The mite thrives under the environmental conditions of 23.2 ± 2.1 °C and 71 ± 5.6% RH (Adler 2004, Riudavets 2004). Sánchez-Ramos and Castañera (2005) reported that at 30 °C and 90 ± 5% RH, the highest intrinsic rate of natural increase of 0.397, the lowest mean generation time of 12.6 and the lowest population doubling time of 1.75 days were obtained. The mites rapidly accumulate under favorable conditions, leading to a substantial depletion of the commodity thereby causing significant economic losses (Ždárková 1991, Thind and Clarke 2001, Sánchez-Ramos et al. 2007). According to Sánchez-Ramos et al. (2007), 100 mites can render about 100 g of dog food to dust in less than four weeks under ideal conditions.

Pest status of *T. putrescentiae*

T. putrescentiae is a major stored-product pest of commodities of high protein, high fat and/or moisture content of 15 - 45%. It has been reported to be associated with over 140 commodities (Hagstrum et al. 2013) including dried fruits, spices, cultured cheeses, and other high value foods (Rentfrow et al. 2006). The mite is also the most abundant and predominant mite species found in processing and packing areas of dry pet food factories (Thind 2005, Nayak 2006, Brazis et al. 2008), cereal based food products and other organic materials that are susceptible to storage mite infestation (Thind and Clarke 2001, Kheradmand et al. 2007). It will

also infest laboratory cultures of insects and microorganisms (Kheradmand et al. 2007). The ham mite has been reported to cause allergic reactions in dogs fed with infested food (Vollset et al. 1986) and in humans exposed to the mite (Vidal et al. 2004, Jeong et al. 2005, Wakefield 2006).

Pest status of *T. putrescentiae* on dry-cured ham

T. putrescentiae is a major pest of southern dry-cured ham during aging. Dry-cured ham, also referred to as country ham, country style ham or country cured ham, is produced from the hind leg of a pig carcass. A typical American dry-cured ham is prepared by the dry application of salt and one or more optional ingredients such as nutritive sweeteners, spices, seasonings, flavorings, and sodium or potassium nitrate (USDA 1999). The curing mix draws out moisture and breaks down the myofibrillar protein to achieve its unique cured flavor. Hence the ham loses at least 18% of its original weight, mostly moisture, during aging and should contain at least 4% salt to be labeled as country cured ham (USDA 1999). Sometimes, the ham is cold-smoked with hardwood such as hickory after curing. Aging is then done at 24 - 35 °C and 55 - 65% RH in aging rooms for up to two years. These environmental conditions are favorable for the growth and development of *T. putrescentiae* (Graham et al. 2011). There is little or no infestation when the ham is aged for less than three months. The risk of infestation however increases with ham aged for more than five months. However, short aging duration of less than five months does not assure processors that infestation problems would not occur (Rentfrow et al. 2006) and aging times longer than six months are necessary to obtain the desired product flavor and quality that meets the niche markets (Toldrá et al. 1997).

Management of *T. putrescentiae*

Many mite pest management practices have been studied and/or implemented to help prevent and/or control ham mite infestations. Chemical methods include the use of fumigants and acaricides. Other methods are biological and physical methods, and the use of food grade oils, and plant extracts and oils. It should be noted that the future of any alternative management method lies in its ability to be incorporated into a decision-based IPM program. The combination of these methods into IPM could help avoid infestations, minimize impacts on foods susceptible to the mite, and more importantly, delay the development of resistance to one particular control method.

Chemical methods

Chemical control methods are commonly used in stored grain, unfinished food products (Stara et al. 2011) and ham processing facilities (Rentfrow et al. 2008). Registered postharvest fumigants for stored product-pests include methyl bromide, phosphine, and sulfuryl fluoride, while ozone and carbon dioxide are likely allowed in most or all countries.

Methyl bromide has been used to control pests in commodities and buildings worldwide since the 1930s because of its broad spectrum application and rapid toxicity (Fields and White 2002). It is nonflammable and noncorrosive (Bond 1984) and does not leave residues on products. Methyl bromide kills mites, insects, nematodes, and micro flora rapidly by damaging the membrane of nerve cells (Fields and White 2002). Methyl bromide is the only known fumigant effective at eradicating existing ham mite infestations (Rentfrow et al. 2006). Typically, methyl bromide fumigation occurs one to five times per year when used by a ham facility (Rentfrow et al. 2006). With limited availability, ham producers will soon need to rely on

alternative fumigants combined with better management programs to help effectively manage the pest.

Phosphine is considered to be the most widely used fumigant alternative to methyl bromide (Fields and White 2002) due to its easy application, effectiveness, low cost, lack of residues (Zuryn et al. 2008), and its ability to disperse easily during fumigation (Nath et al. 2011). Complete mortality of all life stages has been achieved under laboratory conditions (Zhao et al. 2012). In a simulated aging house that contained ham mite assays and dry-cured ham inoculated with ham mites, 99.8% mortality was recorded two weeks post fumigation (Zhao et al. 2015). In another study, mite colony jars placed in treated and untreated ham aging rooms confirmed high mortality in treated aging rooms. However, massive corrosion to electrical wiring and equipment was discovered in the fumigated room about three weeks after fumigation (Phillips and Schilling 2013). Corrosion to metals is the major drawback that prevents phosphine from being an ideal fumigant in all structures (Price and Mills 1988, Brigham 1998, Adler 2004). If ham aging houses could be well sealed and all electrical devices removed or protected, phosphine could be considered a potential alternative to control mite infestations.

Sulfuryl fluoride has been registered for use in dry-cured ham processing facilities since 2005 (EPA 2005). However, the use of this fumigant under laboratory conditions only resulted in 95% mortality of mite eggs at 20 - 22 °C, even when applied at approximately 100.3 g/m^3, which is three times the label rate (Phillips et al. 2008). More recently, Phillips and Schilling (2013) reported that sulfuryl fluoride may be effective at controlling all life stages of the mite when tested for 48 h at 35 °C under laboratory conditions.

Other fumigants that have shown some potential under laboratory conditions include ozone and carbon dioxide, CO_2. Ozone concentration of 155 ppm for 48 h resulted in the

complete mortality of all stages of *T. putrescentiae* under laboratory conditions (Hassan et al. 2010). However, in the real world applications, ozone may not work well since it does not penetrate surfaces well. The use of CO_2 at high pressure in pressure chambers also offers complete control of mites (Adler 2004, Navarro 2006, Riudavets et al. 2010). Riudavets et al. (2010) treated *T. putrescentiae* with CO_2 at two high pressures, 15 and 20 bars. There was over 99% mortality for mixed stages of *T. putrescentiae* on artificial standard diets when treated with CO_2 at 15 bar for 15 minutes, and 100% mortality when the exposure time was increased to 30 minutes. Although CO_2 may be effective, increasing the pressure may mean an increase in the cost of treatment as more CO_2 is required (Riudavets et al. 2010). Mortality of all stages of mites have been achieved at a concentration of 60% CO_2 with 144 h of exposure (Hassan et al. 2010). However, this may not be practical since the duration of exposure would require that treated facilities remain closed for longer and this would interfere with the operation of the facility.

Many other pesticides have been recommended to control storage mites and a number of them have been shown to be effective on the ham mite. Studies on fenitrothion and pirimiphos-methyl have showed contradicting results (Hartmannova et al. 1973, Chmielewski 1987, Nayak 2006). Other pesticides tested by Nayak (2006) included chlorpyrifos-methyl, deltamethrin, pyrethrin + piperonyl butoxide, s-methoprene and spinosad. Out of these, only pyrethrin + piperonyl butoxide, s-methoprene, and spinosad controlled the mite population after at least three weeks of exposure to treated wheat. In another study, Sánchez-Ramos and Castañera (2000) tested 13 monoterpenes and seven of them including pulegone, menthone, linalool, and fenchone were effective against the mobile stages of *T. putrescentiae* by vapor action. However, none of them were effective against eggs. In a more recent study, seven commercial residual pesticides were tested by exposing mites to treated surfaces. Among the tested pesticides, Centynal®

(deltamethrin), Phantom® (chlorfenapyr) and Storcide II® (chlorpyrifos-methyl + deltamethrin) showed promising control on mite populations (Abbar et al. 2013). Although some pesticides may be effective in controlling mites under laboratory conditions, their use is restricted in ham facilities due to concerns about residues (Sánchez-Ramos and Castañera 2003). One suggestion would be to treat surfaces in aging rooms before the cured hams are transferred to these rooms (Abbar et al. 2013).

Biological methods

There have been contradicting results reported on the biological control of *T. putrescentiae* by the predatory mite *Cheyletus malaccensis* Oudemans (Palyvos and Emmanouel 2004, Cebolla et al. 2009). *Cheyletus eruditus* (Schrank) (Acarina: Cheyletidae) has been reported to control mites in stored grains (Ždárková 1998). The use of biological agents against stored product mites may not be very common because the introduction of natural enemies may contaminate the commodity. Contamination of commodities by natural enemies is as bad as contamination by pests. Federal regulations under the Federal Food, Drug and Cosmetic Act do not distinguish pest fragments contributed by stored-product pests from those contributed by natural enemies. Nonetheless, depending on the commodity, most of the natural enemies can be removed (Hagstrum and Subramanyam 2006).

Physical methods

Physical methods involve the manipulation of the mites' environment to make it less suitable for their proliferation. These methods include manipulating light, temperature, and humidity of the environment. García (2004) investigated some physical methods in five Iberian ham factories in the Iberian Peninsula and reported a reduction in the growth rate of mites on ham surfaces when humidity was reduced to levels below 60%. However, mites were forced to

seek refuge in cavities inside the hams where they could feed and reproduce. Boczek (1991) reported that ham mite eggs are killed within 24 h when exposed to -15 °C but will survive at -10 °C. At -5 °C, a longer exposure of 24 d is required to obtain similar results. Other researchers stated that freezing at -28 °C for 48 h did not kill the eggs (Arnau and Guerrero 1994). A more recent study suggested that 90% of all stages of *T. putrescentiae* can be controlled by freezing to -8 °C for 5 h (Eaton and Kells 2011). Rentfrow et al. (2006) also suggested that hams are frozen to control mites on ham. However, repeated freezing and thawing and high heat may affect the quality of ham.

Although manipulation of the environment may be effective, facility managers may require professional assistance to create and/or maintain the new environment. Environmental conditions that are cost-effective and do not significantly affect the quality and/or organoleptic properties of the products should be investigated.

Food grade oils and preservatives

Food grade oils and other commonly used preservatives "Generally Regarded as Safe" (GRAS) are effective in controlling the ham mite. A commonly used method several decades ago was to rub lard on cured meat to prevent flies and bacteria (Smith 1923). This method is still being used today as coating hams with vegetable oils or hot lard is a common practice in Spain to control mites in dry-cured ham (García 2004), although the aging time may be extended since lard may make the ham less permeable to moisture. Lard has also been shown to be effective on ham mites under laboratory conditions (Abbar et al. 2016). Propylene glycol, an odorless, colorless solvent registered as a bacteriostat and fungistat (EPA 2006) has been tested and reported to be effective in controlling mite infestations by preventing mite reproduction under laboratory conditions (Abbar et al. 2016).

Plant extracts and Oils

The health hazards associated with the use of synthetic chemicals in managing mites may probably have resulted in the search for more selective natural compounds. Lee et al. (2006) compared the acaricidal activity of *Foeniculum vulgare* (fennel) seed oil components with benzyl benzoate, dibutyl phthalate and N, N-diethyl-m-toluoamide against *T. putrescentiae* and obtained promising results. The acaricidal activity of *Pinus pinea* essential oil was also evaluated and reported to elicit complete mortality on adult *T. putrescentiae* (Macchioni et al. 2002).

Integrated Pest Management of *T. putrescentiae*

Several authors have discussed the effectiveness of stand-alone management options in the control of *T. putrescentiae* (Clarke 2002, García 2004, Mueller et al. 2006, Rentfrow et al. 2006). However, very few authors have described the possibility of implementing IPM programs. Rentfrow et al. (2006) proposed the implementation of the hurdle approach within an IPM program which may be able to help prevent ham mite infestations in ham processing plants. Their approach involved the survey and auditing of 20 ham processing plants based on which the authors proposed that immaculate sanitation practices especially in both the processing rooms and the aging rooms should be carried out. Grass should be prevented from growing within 9 m of the aging house and wooden racks and structures sealed to prevent mites from hiding in cracks and crevices. The use of breathable paper bags for the hams as well as freezing hams at six months of aging could also help to keep mites away.

A successful IPM program relies on an effective monitoring system that provides information on pest infestations and their incidence over time, especially before and after an IPM program is implemented (Campbell and Hagstrum 2002, Thind and Ford 2004). However, less research on monitoring has been carried out in mites compared to insects. In most facilities, early

detection is not effective because personnel usually do not have reliable equipment to inspect for and detect mites particularly during the early stages of an infestation or when they are present in fine dust or debris (Thind and Ford 2004). Mite monitoring is usually done by placing baited traps in facilities and inspecting the traps for mites. The BT® trap is the first trap designed specifically for the detection and monitoring of storage mite populations and infestations in food or feed storage and production premises. It has also been used for detecting mites in both laboratory and field studies (Thind and Ford 2004, Wakefield 2006). In stored grain and oilseed, the PC™ trap is effective in detecting storage mites and may provide an early indication of their presence when compared to traditional methods (Clarke 2002, Dunn et al. 2005).

Enhancing trapping and correlating trap capture with other measures of sampling

Several trapping efficiency and improvement studies have been carried out in stored-product insects. Factors that have been considered include trap design, shape, color, placement, and response to attractants and light. Traps with smaller openings reduce excess dust accumulation and are preferred because dust reduces trapping efficiency (Toews and Nansen 2012). Surface traps such as the Dome trap are designed so as to take advantage of the insect's preference for the dark (Toews and Nansen 2012). Nansen et al (2004) evaluated the extent to which trap capture of the Indian Meal Moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) was affected by height and concluded that trap efficiency increased with traps near surfaces such as the ceiling or the floor. Trap capture of the red flour beetle, *Tribolium castaneum* (Herbst) and confused flour beetle, *Tribolium confusum* Jacquelin du Val (both Coleoptera: Tenebrionidae) can be improved by placing pheromone/kairomone baited trap near black pillars against white backgrounds (Semeao et al. 2011). Some compounds identified as

potential attractants for storage mites are fungal volatile 1-octen-3-ol and food volatile 2-nonanone (Dunn 2000). These attractants were tested and reported to increase trap capture of *Lepidoglyphus destructor* (Schrank) (Acari: Glycyphagidae), *Acarus siro* L. (Acari: Acaridae) and *Tyrophagus longior* (Gervais) (Acari: Acaridae) in wheat or oilseed rape when incorporated in BT® traps and PC™ traps (as reported in Wakefield 2006). However, these attractants have not been investigated in *T. putrescentiae*. Research has also shown that pheromone-alone traps only detect a small percentage of pests present. Trap capture of *T. castaneum* in a pheromone-alone Dome traps was improved from 1% to 20% by attaching an ultraviolet (UV) Light Emitting Diode, LED (Duehl et al. 2011).

There has also been no reported study to compare and or correlate ham mite sampling techniques, or develop sampling plans, although many of such studies have been carried out in stored-product insects (Hagstrum et al. 1985, Pereira et al. 1994, Subramanyam et al. 1997, Toews et al. 2003, Savoldelli 2006, Buckman and Campbell 2013).

Relevance of study and objectives

Few studies towards the IPM of *T. putrescentiae* have been carried out probably because the control of this mite had not been an issue until the ban of methyl bromide (EPA 2006). The development and implementation of IPM programs for the ham mite is therefore still in its infancy. For some time now, ham producers in the United States fumigated when mite infestations were visually observed (S. Edwards, personal communication). Many producers do not have a mite monitoring plan to detect or estimate mites to decide if the mite population is high enough to warrant control. Some producers have a scheduled fumigation and would fumigate their facilities even when populations may not warrant control (Amoah et al. 2016). As

a result of ineffective monitoring procedures, high levels of infestations may go unnoticed for long periods of time until mites "suddenly" appear. With the ban of methyl bromide, there is the need for monitoring plans so that other alternatives can be developed for the ham mite. In many stored-product pests, the aim is to detect infestations early and improve trap capture so that monitoring will be a better representative of the actual density of the pest. Several factors have been determined to enhance trap capture in stored-product insects but not as many in stored-product mites. There may be low levels of mites found in baited traps, even when there is a high level of infestation in, on or around commodities. Mite dispersal characteristics may therefore influence the efficacy of monitoring procedures hence, correlating trap capture with other direct measures could help give a more accurate representation of the population density of the mite and therefore determine when control measure is required. This study was focused on detecting and monitoring ham mite populations under various conditions, correlating mite sampling techniques, and developing sampling plans for the mite.

Objectives

1. To implement and use food-baited prototype traps to monitor ham mites in three commercial ham processing facilities. Traps were used as a relative, indirect sampling method to help determine the population dynamics of the mites in the facilities over a period of about a year.
2. To determine factors affecting orientation to food baited traps. Factors that were investigated include trapping duration, trap distance from mite source, trap design, and light of different wavelengths.

3. To compare and correlate sampling techniques with actual mite densities and develop sampling plans for the mite. The sampling techniques that were studied included trapping, vacuuming and direct counts on ham.

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Chapter 2 - Monitoring *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) with traps in dry-cured ham aging rooms

Abstract

Methyl bromide is the most effective fumigant for controlling the mold (or ham) mite, *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae), the most significant pest of dry-cured ham. However, methyl bromide is being phased out of use. Therefore, integrated pest management (IPM) methods should be developed to help control mites in dry-cured ham plants. The foundation of a successful IPM program is an effective monitoring program that provides information on pest presence and abundance by location and over time. By using food-baited traps fabricated from disposable Petri dishes and a dog food-based bait, mite activity over time and space was monitored in five dry-cured ham aging rooms from three commercial processing facilities that differed in their fumigation frequencies. Weekly sampling of the mite was conducted from June 2012 to September 2013. There were significant differences in the average weekly trap captures in all facilities, especially before and after fumigation, with the majority of mites in traps prior to fumigation. Mite numbers had a pattern of sharp decline after fumigation, followed by a steady increase until the next fumigation. Average trap captures varied due to trap location over the study period at all study sites, indicating that traps could be used to identify specific locations within an aging room where mite infestation of hams was more likely to occur. These findings can inform facility managers of mite population changes that can be used as one factor towards making pest management decisions and assessing the impact of fumigation or other pest mitigation actions.

Introduction

Tyrophagus putrescentiae (Schrank) (Acari: Acaridae) is a pest of dry-cured ham, aged cheeses and other post-harvest products (Boczek 1991). Southern dry-cured ham is produced from the hind leg of a pig carcass. The ham is first cured by rubbing salt and other seasonings on the ham and storing under refrigeration conditions (2 - 4 °C) for 4 - 6 weeks to allow salt to penetrate through the ham by osmotic pressure, which preserves the ham. After curing, hams may be smoked with hardwood such as hickory or left unsmoked. Hams are then hung on racks and aged at 24 - 35 °C and 55 - 65% relative humidity (RH) for a minimum of two months and sometimes up to two years (Graham et al. 2011). During aging, the loss of moisture, proteolysis, and lipolysis result in a unique flavor and taste (Toldrá et al. 1997). Long-aged hams have a more complex and intense flavor that is appreciated by some consumers (Marriott and Schilling 2004). However, hams aged for more than five months are more likely to be infested *with T. putrescentiae* (Rentfrow et al. 2006), commonly known as the ham mite. *T. putrescentiae* has been isolated from over 140 commodities (Hagstrum et al. 2013), most of which are of high protein and/or high fat content with a moisture content of 15 - 45%. These commodities include dried fruits, spices, dried meats, cultured cheeses (Rentfrow et al. 2008), pet food (Thind 2005, Brazis et al. 2008), cereal-based food products and other organic materials (Thind and Clarke 2001). This mite causes direct damage that reduces product quality and quantity and may result in product rejection (Jeong et al. 2005). *T. putrescentiae* is sometimes considered a member of the house dust mite complex and has been reported to generate human allergic reactions (Jeong et al. 2005, Wakefield 2006). The optimal growth of *T. putrescentiae* occurs at environmental temperatures of 23.2 ± 2.1°C and RH of 71 ± 5.6% (Adler 2004, Riudavets 2004). According to Sánchez-Ramos et al. (2007), a mite population may double in as little as two to four days.

Under ideal conditions, 100 mites can render about 100 g of dog food to dust in less than four weeks.

The BT® trap was the first trap designed specifically for the detection and monitoring of storage mite populations and infestations in both laboratory and field studies (Thind and Ford 2004, Wakefield 2006). The BT® trap is not truly a trap but rather a food-bait station. The trap is a small black disc that contains a moist food bait and is equipped with openings around the entire circumference of the base to allow access by mites. In a study conducted by Thind and Ford (2004), 15 - 25 BT® traps were used to detect and monitor storage mites in three factories that produced cereal-based foods for dogs, small animals and horses, or moist, meat-based pet food for dogs and cats. Mites included *Tyrophagus* spp, with the most predominant being *T. putrescentiae*. Although numbers were low, the study showed that the traps were effective at capturing mites, even after professional cleaning and sanitation had been carried out. In a comparative field trial study conducted by Thind (2005), the BT® trap detected more mites than a food-bait bag composed of a mixture of cereal, peanut, and carob, or a fishmeal trap, made of a moist filter paper with fishmeal covered with a raised Petri dish in four different types of premises. These premises included three factories involved in the production of cereal-based dry pet food, specialist animal feed, traditional cheese, and in the finished cereal section of a large transit storage warehouse. At least seven species of storage and predatory mites were detected in these locations. Wakefield and Dunn (2005) reported that under test conditions of 20 °C and 65% RH, the BT® trap was effective at detecting mites for at least 10 days for all three species tested: *Lepidoglyphus destructor* (Schrank), *Tyrophagus longior* (Gervais) and *Acarus siro* (L.) (All Acari: Acaridae). Individuals of these three species reached a trap at a distance of approximately 80 cm in a 24 h period. In addition to the BT® trap, The PC™ trap, which is used to monitor

storage insects in bulk-stored grain, was effective at detecting mites in stored grain and oilseeds (Clarke 2002). According to Dunn et al. (2005), the BT® and PC™ traps were more effective at detecting low numbers of storage mites, including *A. siro*, *T. longior*, and *L. destructor* when compared with sampling and sieving.

Fumigation with methyl bromide has been the most effective method to control mite infestations in dry-cured ham plants. Methyl bromide fumigation may be performed one to five times per year in some dry-cured ham facilities in the United States (Rentfrow et al. 2008). However, methyl bromide use is being phased out of use as part of the Montreal Protocol, due to its ozone-depleting nature (EPA 2014). Dry-cured ham producers in the United States need to identify newer methods to control mite infestations. Integrated pest management (IPM) programs need to be developed for ham mites on dry-cured ham. Monitoring ham mites to estimate their population density relative to commercial action levels is essential in developing an IPM program for dry-cured ham. Many ham processing facilities do not have systematic monitoring programs in place, so infestations may go unnoticed for long periods of time.

The broad objective of the work described below was to determine if a simple food-baited trap could be used to detect and monitor *T. putrescentiae* populations. Monitoring mite activity over time and across spaces in a given structural habitat could give insights on mite population dynamics and therefore help in developing IPM programs. A specific objective was to determine if the food-baited trap could confirm the impact of methyl bromide fumigations on mite populations at commercial ham processing and aging facilities.

Materials and Methods

Trap design and food bait

Large numbers of BT® traps were not available for purchase for this study, which motivated the development of a low-cost, easily constructed trap. The food-baited trap that was developed in the Kansas State University Stored Product Entomology Laboratory was a plastic Petri dish, 100 mm diameter x 15 mm high that was spray-painted black on all outside surfaces. Eight evenly-spaced holes, each of approximately 0.5 mm diameter, were made above the bottom along the side of the dish, approximately 2 mm from the bottom, to allow mites to enter the trap. A 10 g circular disc of the food bait, approximately 30 mm diameter and 10 mm high, was placed inside at the center of the dish bottom. The lid and bottom of the Petri dish were held in place using a rubber band. Preliminary testing of the food-baited traps indicated that mites enter the traps through the entrance holes to feed, mate, and lay eggs on the diet plug. Although mites were not physically retained in the traps, and could move freely in and out of the traps, it was observed that mites would clearly aggregate at the food plug once they encountered it.

Food baits for traps were made prior to each weekly trap deployment in batches of approximately 600 ml with the following ingredients: 475 ml water, 160 g of dry dog food (a retail commercial product composed mainly of yellow corn, chicken by-product meal, beef, rice and soy), 40 ml glycerol (99.9%) (Fisher Scientific International, Inc.), 5 g brewer's yeast (MP Biomedicals, LLC), 10 g Agar (LCN Biomedicals, Inc.), 5 g alphacel (non-nutritive bulk cellulose) (LCN Biomedicals, Inc.), 5 g insect vitamin mix (MP Biomedicals, Inc.), and 5 ml of methyl-p-benzoate solution (15% methyl-p-benzoate in 95% ethanol) (LCN Biomedicals, Inc.). The dry dog food pieces were soaked in the water to soften and then blended for approximately 90 s at the highest setting in a kitchen blender until smooth with no apparent separate food

pieces. The smooth mixture was combined with glycerol in a 1000 ml Pyrex glass beaker and then brought to a boil before adding the remaining ingredients, after which the mixture was allowed to boil for approximately five minutes more to ensure the ingredients are completely dissolved and to activate the agar. The mixture was then poured into a stainless steel rectangular pan measuring 25 cm x 15 cm x 5 cm, allowed to cool to room temperature and covered with aluminum foil. This was then stored in the refrigerator until needed for traps, at which time the circular discs were cut from the gelled food using a 30 mm diameter cork borer.

Ham aging rooms

Pest populations of *T. putrescentiae* were monitored in three commercial facilities (A, B, and C) located in the Southeastern United States that manufactured southern dry-cured hams. Indoor temperature and humidity of each facility was monitored and recorded from April to August 2013 using data loggers (HOBO U12-006, Onset Computer Corporation, Pocasset, MA). At least one data logger was used per facility. Outdoor temperature and RH data were obtained for the same period from weather stations located within five miles of each of the facilities. Trapping was conducted between June 2012 and September 2013. The three facilities varied widely in their sizes, number of hams in aging rooms at any given time, and pest management procedures. Physical descriptions of aging rooms are given below.

Facility A. Two aging rooms in Facility A were used for this study (Figure 2.1). Although both rooms were in the same building, they were separated from each other by 180 m and several rooms. Room 1 was approximately 24 m long, 11 m wide, and 4 m high and contained approximately 10,000 hams when full, while room 2 was approximately 15 m long, 9 m wide, and 3 m high with approximately 7,000 hams when full. Hams were hung on wooden

racks throughout the aging period. Hams in room 1 were typically aged from two to four months and those in room 2 were aged up to 24 months. Hams in both rooms were moved in and out all year round as needed by the company. The humidity was usually kept at 50 to 88% for both rooms. Traps labeled 1-10 were placed in room 1 and traps 13-19 were placed in room 2. Traps 11 and 12 were located just outside room 1, and trap 20 was placed in the loading dock area. Methyl bromide fumigations were performed on a given weekend for 24 h, with preparation on a Friday and ventilation on a Sunday. Fumigation with phosphine (hydrogen phosphide gas) was done in two cases during the study period.

Facility B. Two aging rooms, one in each of two separate buildings, were used at Facility B (Figure 2.2). Room 1 was approximately 18 m long and 5 m wide with steel racks while room 2 was approximately 14 m long and 8 m wide with wooden racks. Temperature and humidity were maintained at approximately 25 ± 1°C and 45-58% respectively. Traps 1-9 were deployed in room 1 and traps 10-20 were deployed in room 2 for the majority of the study period from week 26 to week 59. Prior to week 26, trap locations changed two different times: week 6, and week 11. Facility B was fumigated three times during the study period and these were done usually upon recommendations after trapping results. Both rooms were fumigated on Friday of week 5 because of the density of mites, but the other two fumigations in weeks 28 and 53 were carried out only in room 2.

Facility C. Facility C was the largest building of the three facilities studied. The aging house used for the study measured approximately 55 m long and 14 m wide with wooden racks. There were 19 small aging rooms in the aging house, each measuring approximately 5 m long and 5 m wide. Typically, the age of hams ranged from one to six months. Each small room contained hams of the same age at any point in time. One trap was placed in each of the 19 small

rooms and the trap labeled 20 was placed in the common area of the aging house (Figure 2.3). There was no change in trap locations throughout the study period. Four methyl bromide fumigation exercises were carried out during the study period. The first was just before the start of the study and then on the Fridays of weeks 5, 12 and 52.

Trap deployment and evaluation

Preliminary testing of the food-baited traps indicated that one week was adequate for detecting the presence of mites. Twenty traps were shipped to each of the three ham facilities on a weekly basis so they could be deployed on Mondays for a seven-day period, after which traps were collected, shipped back, and replaced with the new traps. After each one-week period of deployment, individual traps were placed in a plastic sandwich bag by facility cooperators and returned to the laboratory for evaluation. Traps were deployed no closer than 1 m and not farther than 8 m apart on the floor along the walls, near machinery or other structural edges, or underneath racks in darkened locations in Facilities A and B. Traps in Facility C were placed on exposed ledges approximately 1 m above the floor. Selected trap locations were unlikely to be disturbed by the operations of the facilities. Data were scored as the count of all mobile mites inside the dish at the end of a given trap deployment period. Eggs were not counted. The same trap placement locations were maintained in each facility throughout the study unless otherwise indicated. Fumigation, including preparation, gas exposure and ventilation, was normally from Friday to Sunday and the facilities re-opened for business on Monday. The traps were collected at the end of the week immediately preceding a fumigation.

Data processing and analysis

Data are presented as the mean number of mites per trap with the standard error of the means calculated for each week and for each facility. Similarly, the proportion of traps with and without mites in a given week was also calculated. When possible, the mean number of mites per week captured at each trap location within an aging room was calculated to assess variation among trap locations. The variation in numbers of mite captures over consecutive trapping periods for each of the five aging rooms was evaluated. To rule out the effect of fumigation during an assessment period, trapping periods of 10 weeks or more for which the first week was that just after a fumigation and the last week was that just before the next fumigation were selected. Facility A was not included in this analysis due to its high frequency of fumigation not meeting the criterion of 10 weeks between fumigations. Thus, one trapping period was assessed for room 1 and two trapping periods were assessed for room 2 in Facility B; one trapping period was used for Facility C. The correlation of mean mites per trap, the proportions of traps with mites and mean mites per week with trapping periods in the various rooms were calculated as described using PROC CORR (SAS Institute 2010). Differences in mite capture by trap location across weeks were analyzed for each room using PROC GENMOD (SAS Institute 2010) such that each trap location was an experimental unit deployed in a completely randomized design. The probability distribution was set to a negative binomial and the link function was set to LOG transformation since initial analysis determined that the data were not normally distributed. The means were separated on the basis of the Tukey-Kramer's Honestly Significant Difference Test.

Results

T. putrescentiae were captured in traps within all three commercial facilities throughout the study period. Results indicated that ham mites could be detected inside infested ham aging rooms, and that the weekly numbers in traps before and after fumigations reflected the changes in relative mite population sizes over time in a given aging room (Figure 2.4, Figure 2.5, Figure 2.7, Figure 2.8, and Figure 2.10). Indoor temperatures and RH for the three facilities are presented in Table 2.1. Trap capture over time showed a clear reduction in the numbers of mites immediately following methyl bromide fumigation for nearly every case in all the aging rooms studied, but effects of cleaning on mite numbers at one facility were not clear-cut (Figure 2.4, Figure 2.5, Figure 2.7, Figure 2.8, and Figure 2.10). Trapping data are presented below for each facility separately.

Facility A

In room 1 of Facility A, trap captures in the week following a given fumigation ranged from zero to approximately 25 mites per trap. For instance, there were no mites in any trap for the first week after the fumigation, while the count after the week 25 fumigation was 24.7 ± 12.6 mites per trap (Figure 2.4). Of all the fumigant applications, only the fumigation carried out during week 32 in room 2 resulted in zero trap counts for four consecutive weeks after fumigation. The percentage of traps with mite captures for both rooms during the study period ranged from 0% to 100% Figure 2.4 and Figure 2.5). Traps were ranked with respect to the mean number (± SE) of mites caught per week by each trap. As shown in Figure 2.6, the trap with the highest average capture was trap number 5 with 29.8 ± 7.7 mites per week, while trap 14 captured the lowest number at 0.5 ± 0.3 mites per week. No trap recorded zero mites throughout

the period of study in Facility A. Also, no trap recorded mite(s) every week throughout the period of study. Traps in room 1 caught mites in 16% (trap 1) to 54% (trap 10) of the trapping weeks. In room 2, traps caught mites between 10% (trap 14) to 37% (trap 19) of the time. Both traps 14 and 19 also recorded the lowest and highest mean number of mites respectively (Figure 2.6). The proportion of weeks for which a particular trap caught mites in Facility A was positively correlated with the mean mites per week at a given trap location (Table 2.2).

Facility B

Fumigation carried out during week 5 in July 2012 kept the trap count below two mites per trap for twenty weeks in room 1 (Figure 2.7) and for six weeks in room 2 (Figure 2.8). Fumigation of room 2 during week 28 reduced the average trap count from 17.4 ± 4.2 to 0.4 ± 0.2 mites per trap. However, the trap counts increased dramatically to 52.8 ± 13.7 mites per trap after only two weeks (Figure 2.8). Up to about 90% of the traps deployed in room 1 (Figure 2.7) and up to 100% of the traps in room 2 (Figure 2.8) captured mites during one or more weeks. There was a positive and significant linear relationship between the trapping period from week 6 to week 60 and the mean mites per trap in room 1. For room 2 there was also a significant positive linear relationship for mites per trap and the trapping period of week 6 to week 28, but a very weak and non-significant relationship between these in the second trapping period of week 29-53 (Table 2.3).

Trap ranking in Facility B showed that the trap number 2 recorded the highest number of mites per week at 97.7 ± 18.5, while trap number 8 recorded the lowest number at 3.7 ± 2.2 mites per week (Figure 2.9). Similar to Facility A, no trap recorded zero mites every week throughout the period of study. Also, no trap recorded mite(s) for each week throughout the period of study.

In room 1, traps captured mites in 18-76% of the monitoring period, for traps 8 and trap 5, respectively. In room 2, mites were caught 24-79% of the monitoring periods for traps 20 and 17, respectively. Traps 20 and 17 also recorded the lowest and highest number of mites respectively as shown in Figure 2.9, and this reflects the positive and significant correlation between the proportion of weeks for which a particular trap caught mites and the mean mites per week at a given trap location for both rooms (Table 2.2).

Facility C

Facility C had a relatively low mite population with an average trap capture of less than one mite per trap for approximately half of the duration of the study (Figure 2.10). The highest trap capture was recorded in week 59 at 18.3 ± 11.3 mites per trap. Average trap count just before the week 5 fumigation was 1.0 ± 0.5, but after fumigation there was an unexpected increase in trap capture to 3.8 ± 3.7 mites. Also, there was a slight increase in the average trap counts after the week 12 fumigation from 6.2 ± 4.6 in week 12 to 7.5 ± 6.1 in week 13. Mite numbers in traps dropped to zero for three consecutive weeks after week 52 fumigation before the average count increased to 15.0 ± 9.7 at week 56. Percent of traps with at least one mite ranged from 5% to 45% and there were 15 weeks during which no mite was captured in any of the traps deployed (Figure 2.10). There were no significant correlations between week after fumigation and either mites per trap or percentage of traps with mites ($P > 0.05$; Table 2.3).

When traps were ranked, the highest number of mites per week was recorded in trap number 11, with 11.0 ± 5.6 mites. Moreover, no mites were caught in trap 15 throughout the entire study period and trap 12 contained mites for only 30% of the monitoring period (Figure 2.11). The

proportion of weeks for which a particular trap caught mites was positively and significantly correlated with the mean mites per week at a given trap location (Table 2.2).

Discussion

The results of this study suggest that the simple, food-baited trap that was developed is able to detect mites and monitor their populations under commercial settings. Up until the beginning of this project, there were no methods available for detecting and monitoring mite populations in ham facilities other than simple visual observations conducted by employees at dry-cured ham facilities, utilizing their own criteria. This study shows that the trap can provide an early warning of a mite infestation which may not be noticed through visual inspections. Therefore, traps can help determine when a treatment or a corrective action is required to control mites. Mites were detected inside infested ham aging rooms, and the numbers of mites in traps apparently reflected the relative sizes or activity of different mite populations relative to the conditions of various locations in the facilities and across facilities. Compared with the use of pheromone traps for stored product beetles and moths (reviewed in Toews and Nansen 2012), only a few studies have been published on the use of baited traps to monitor stored product mites, including *T. putrescentiae* (Thind and Ford 2004, Thind 2005, Wakefield and Dunn 2005, Wakefield 2006). However, this information is critical for the development of IPM programs (e.g., Campbell et al. 2002).

Results suggest that the impact of fumigation on ham mite populations can be monitored with the trap that was developed in this study. Mite captures in traps gave useful information on relative mite numbers before fumigation with methyl bromide, when numbers were high, to directly after treatment when numbers typically dropped to zero for one to several weeks. Trapping is therefore useful to monitor mites before and after fumigation to help determine the effectiveness of the fumigation exercise. Some exceptions to that conclusion in this current study data may be due to either ineffective fumigations, or from an unfumigated source of mites,

perhaps from an adjoining space or cracks and crevices either in racks or other indoor structures (Thind 2005, Wakefield and Dunn 2005), as a source of mites that invaded the treated room after fumigation, causing rebound or gradual post-fumigation increases in mite populations (Campbell and Arbogast 2004). *T. putrescentiae* has a very high intrinsic rate of natural increase, r of up to 0.4-fold per day depending on the food source, temperature and RH (Sánchez-Ramos and Castañera 2005, Bahrami et al. 2007) compared to other stored product arthropod pests such as the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), with 'r' of 0.06 per day at 30 ºC (Kangmontree 2005). *T. putrescentiae* survivors after fumigation could therefore reproduce and disperse, but surviving populations may not have been high enough to be detected in traps until after three weeks in Facility A and up to seven weeks in Facility B following fumigation.

Mite populations may also rebound quickly to pre-fumigation population levels if less than the recommended methyl bromide dosage or a fumigant other than methyl bromide is used. In either case, a significant proportion of a population could remain to reproduce. For instance, half of the recommended dosage of methyl bromide was used by the management of Facility B for the week 28 fumigation of room 2 (Figure 2.8). The average trap count was 0.4 ± 0.2 mites during the first week after fumigation and this increased substantially to 52.8 ± 13.7 mites per trap after two weeks. It was therefore presumed that a longer exposure time or higher gas concentration may have been required for a more effective fumigation exercise. During fumigation, pest mortality is a function of concentration of that gas, the time exposed to that concentration and the temperature of the space under fumigation (Phillips et al. 2012). Therefore it is possible that this room received a sub-optimal fumigation due to deficiencies in any one of these variables. This assumption of a poor fumigation, which resulted in a population rebound is validated since another fumigation at the same facility with the required concentration of

fumigant during week 5 kept the trap counts below two mites per trap for at least six weeks. In Facility A, trap counts typically remained at zero for an average of two weeks after fumigation. However, an average of 15.3 ± 6.7 mites was caught in traps deployed in week 26, a week after the phosphine fumigation exercise in January 2013. This one-week rebound may be due to phosphine being used. Although there have been reports indicating that phosphine may not be as effective as methyl bromide at eradicating mites (EPA 2008), other studies (Mueller et al. 2006, Zhao et al. 2015) reported that all life stages of the ham mite are killed by phosphine. The incomplete kill resulting from this phosphine fumigation exercise may therefore have been due to poor application processes.

In all five aging rooms studied, trap capture varied significantly with the location of traps Figure 2.6, Figure 2.9, and Figure 2.11). Studies have shown that factors such as proximity of trap to the pest (Mullen and Dowdy 2001, see Chapter 3) and presence or absence of food (Toews and Nansen 2012) have an effect on capturing storage pests in traps. Nansen et al. (2004) and Toews and Nansen (2012) recommend placing traps along walls, in corners and/or near the ground as this may increase trap efficiency. In this study, traps were placed on the floor along the walls, near machinery or other structural edges, or underneath racks or on ledges approximately 1 m above the floor. Differences in trap capture may therefore have been due to the proximity of the traps to a mite or food source and/or differences in the microclimate at trap locations.

Sanitation is an important preventive component in any successful IPM program. Excellent sanitation could therefore impact the population dynamics of *T. putrescentiae* and potentially contribute to low trap counts. Also, sanitation can immediately increase trap capture as an increase in travel distance for food increases the likelihood of a pest being captured in a trap (reviewed in Toews and Nansen 2012). Although details of the sanitation procedures in all

the facilities were not documented, a thorough cleaning and sanitation exercise was documented during week 25 in Facility A. Slightly low trap counts in weeks after that exercise could not be attributed entirely to cleaning because phosphine fumigation was carried out during that same week. A brief cursory inspection of Facility C was conducted and the facility was expected to have relatively higher mite numbers than others based on the presumed level of sanitation. However, that was not the case as trap counts were quite low in that building. Concrete explanations about the role of either sanitation or structural characteristics related to numbers of *T. putrescentiae* in traps will require further research. However, it has been reported that aging time of the hams have a greater impact on mite infestations than sanitation of the facilities (Zhao et al. 2016)

This study showed that a mite trap made using a disposable Petri dish and a dog food-based bait was effective at detecting ham mites and monitoring their population dynamics in ham processing facilities. Mites caught in traps varied from zero to several hundred with some areas of the facilities having higher mite activity than other areas. Traps confirmed that fumigation in certain circumstances significantly reduced mite populations. It was also confirmed that mite activity slowly increases after fumigation. Based on this research, mite traps can be distributed in ham facilities and other processing and retail facilities to help monitor mite activity over time and space. Traps can be used in an IPM program to indicate increases in mite populations to plant employees. This could help dry-cured ham plants determine if corrective actions such as fumigation are needed as well as help assess the impact of corrective actions, such as fumigation on mite control.

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

Table 2.1 Temperature and relative humidity recorded in the three ham facilities from March 2013 to September 2013

Company	Room	Indoor Temperature ± SE (°C)			Indoor RH ± SE (%)		
		Low[a]	Average[b]	High[c]	Low[a]	Average[b]	High[c]
A	1	22.9 ± 0.8	24.5 ± 0.8	26.7 ± 0.8	35.7 ± 2.2	43.0 ± 2.8	45.5 ± 2.5
A	2	18.7 ± 1.0	19.8 ± 0.9	20.7 ± 0.9	59.6 ± 1.8	62.4 ± 1.2	65.0 ± 1.1
B	1	25.2 ± 0.8	26.0 ± 0.7	26.6 ± 0.6	51.2 ± 0.7	53.6 ± 0.6	55.8 ± 0.6
B	2	24.5 ± 0.8	24.8 ± 0.8	25.9 ± 0.6	53.3 ± 1.1	55.7 ± 0.8	59.5 ± 0.9
C	NA	16.0 ± 0.5	19.5 ± 1.0	22.9 ± 1.3	56.0 ± 3.8	70.1 ± 3.5	83.2 ± 2.2

[a] The average of all the lowest values for each day

[b] The average for all the values for the entire period

[c] The average of all the highest values for each day

Table 2.2 Correlation of mites per week and proportion of weeks with *T. putrescentiae* in a given trap in five different dry-cured ham aging structures.

Company	Room	n	Correlation (*r* and *P*- values)*	
A	1	12	$r = 0.66$	$P = 0.02$
A	2	8	$r = 0.87$	$P < 0.01$
B	1	9	$r = 0.76$	$P = 0.02$
B	2	11	$r = 0.90$	$P < 0.01$
C	NA	20	$r = 0.64$	$P < 0.01$

*Correlation analyses performed with PROC CORR in SAS v. 9.3 (2010)

Table 2.3 Correlation between trap capture data for *T. putrescentiae* with weekly time periods following fumigation in three different commercial dry-cured ham aging structures.

				Correlations of mite trap data with time after fumigation*			
Company	**room**	**Period of weeks**	**n**	**Mean mites per trap**		**% of traps with mites**	
B	1	Week 6 to week 60	55	$r = 0.41$	$P < 0.01$	$r = 0.53$	$P < 0.01$
B	2	Week 6 to week 28	23	$r = 0.63$	$P < 0.01$	$r = 0.75$	$P < 0.01$
B	2	Week 29 to week 53	25	$r = 0.18$	$P = 0.39$	$r = 0.24$	$P = 0.24$
C	NA	Week 13 to week 52	32	$r = 0.31$	$P = 0.08$	$r = 0.23$	$P = 0.20$

*Correlation analyses performed with PROC CORR in SAS v. 9.3 (2010)

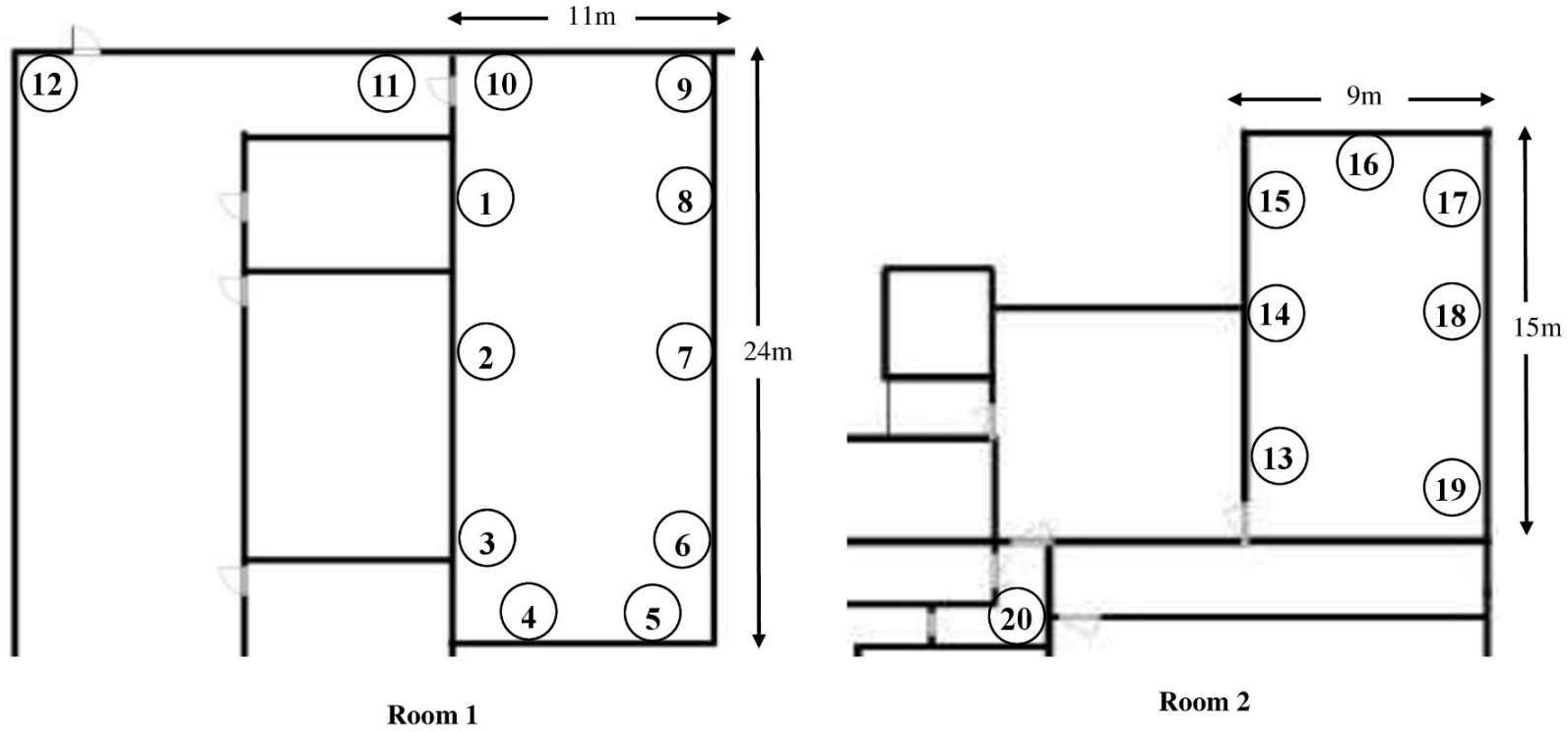

Figure 2.1 Maps of dry-cured ham aging rooms 1 and 2 in Facility A showing trap locations circled.

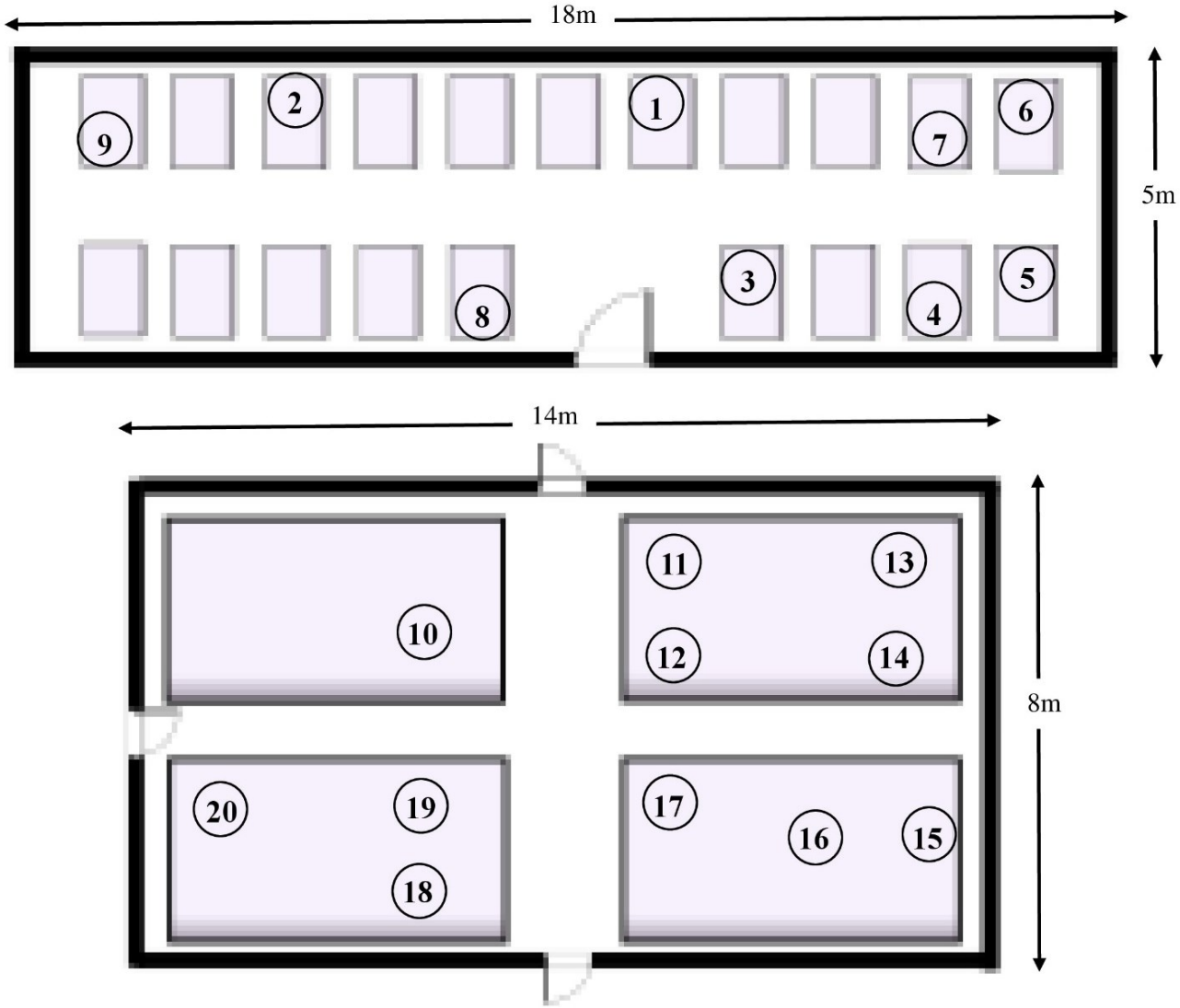

Figure 2.2 Maps of dry-cured ham aging room 1 (above) and room 2 with racks in Facility B showing trap locations circled.

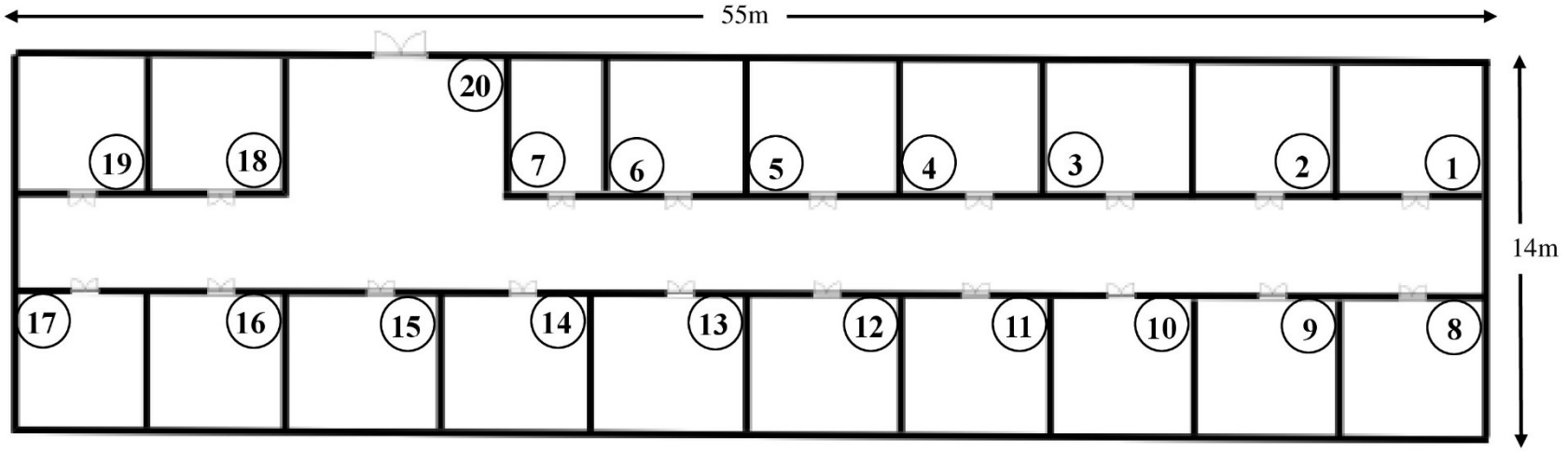

Figure 2.3 Map of dry-cured ham aging rooms in Facility C showing trap locations circled.

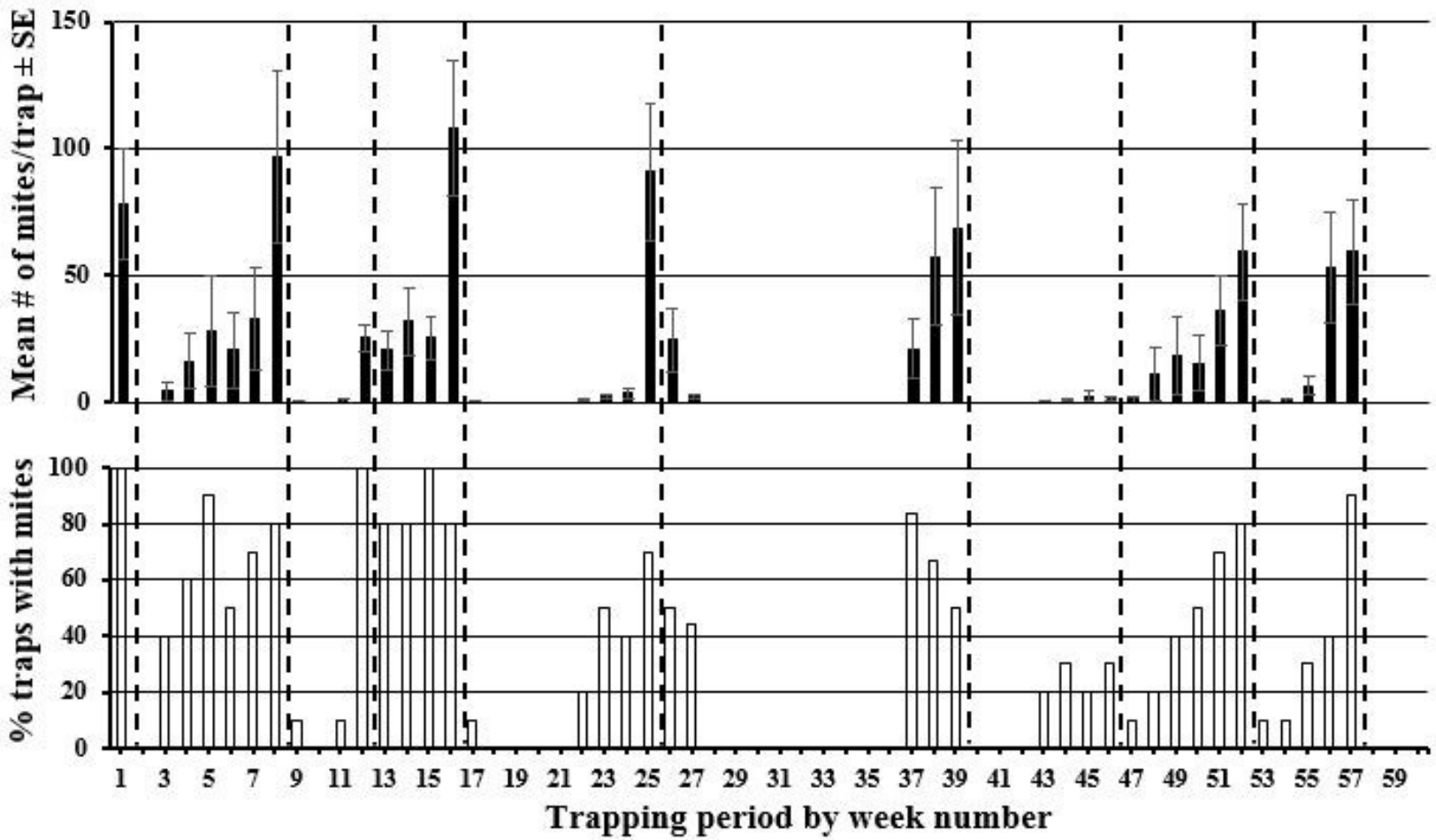

Figure 2.4 Illustration of the average weekly trap captures (above) and percentage of traps with *Tyrophagus putrescentiae* for Facility A Room 1.

The study was from June 2012 to September 2013. The dashed lines indicate fumigation dates. A cleaning exercise was carried out during week 25. Traps were not deployed from weeks 28-36 and 40-42 at the request of the owner.

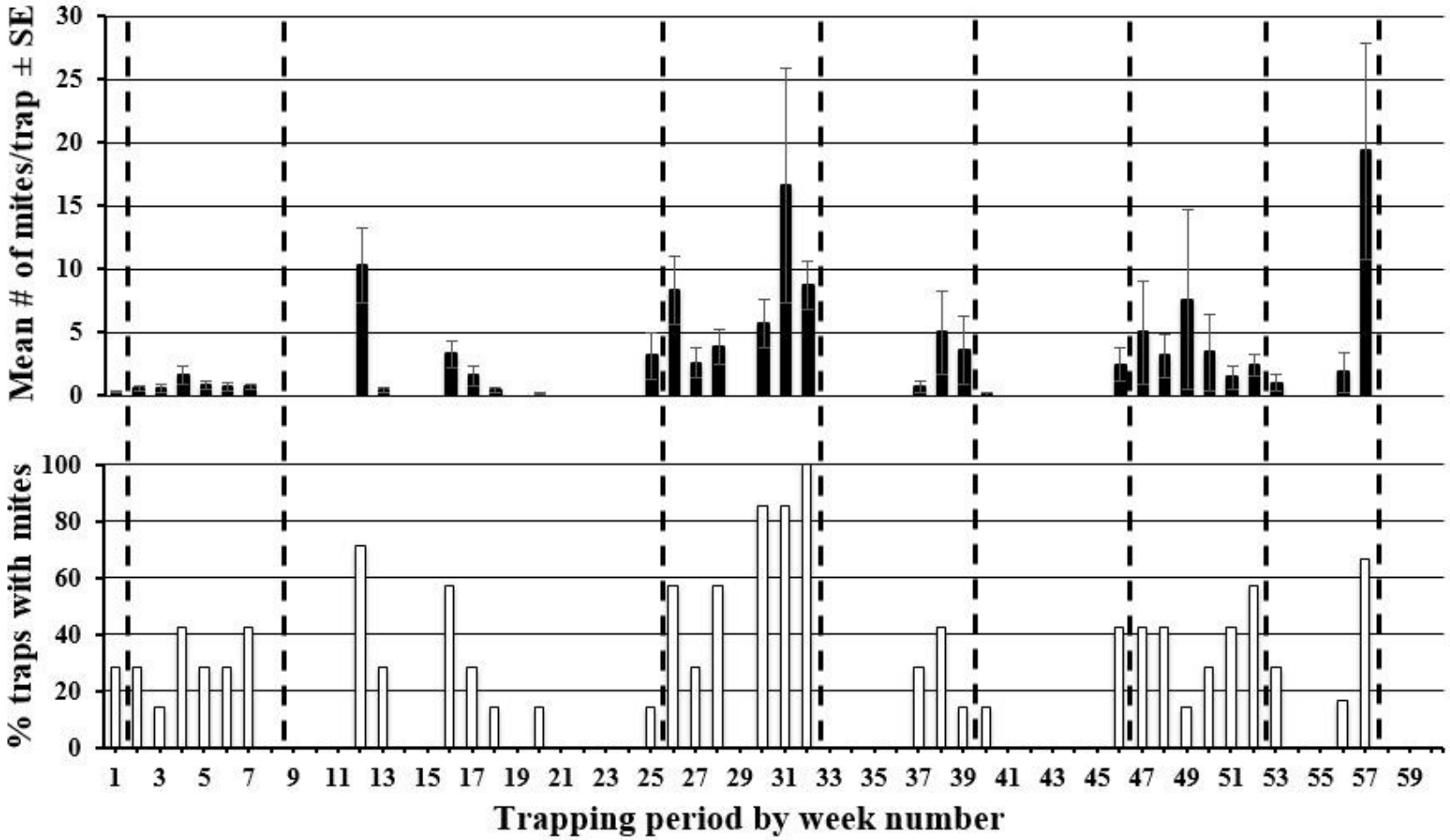

Figure 2.5 Illustration of the average weekly trap captures (above) and percentage of traps with *Tyrophagus putrescentiae* for Facility A Room 2.

The study was from June 2012 to September 2013. The dashed lines indicate fumigation dates. A cleaning exercise was carried out during week 25.

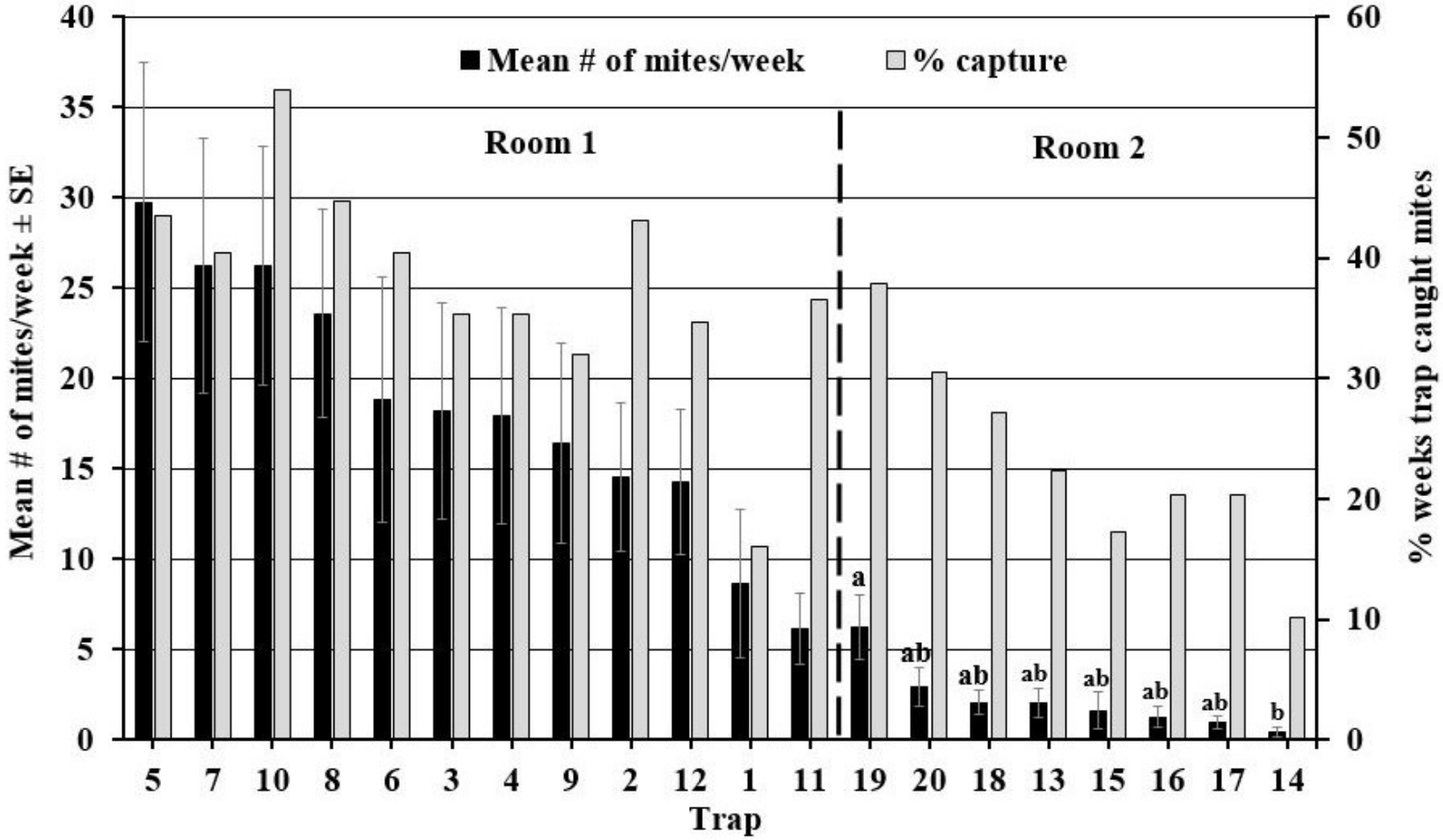

Figure 2.6 Trap ranking using mean (± SE) number of mites per week caught by each trap (left vertical axis) and percent trap capture (right vertical axis) in Facility A.

Trap ranking in two aging rooms (separated with dashed line) for up to 59 weeks. Means with different letters are significantly different ($P < 0.05$; by Tukey-Kramer's Honestly Significant Difference Test).

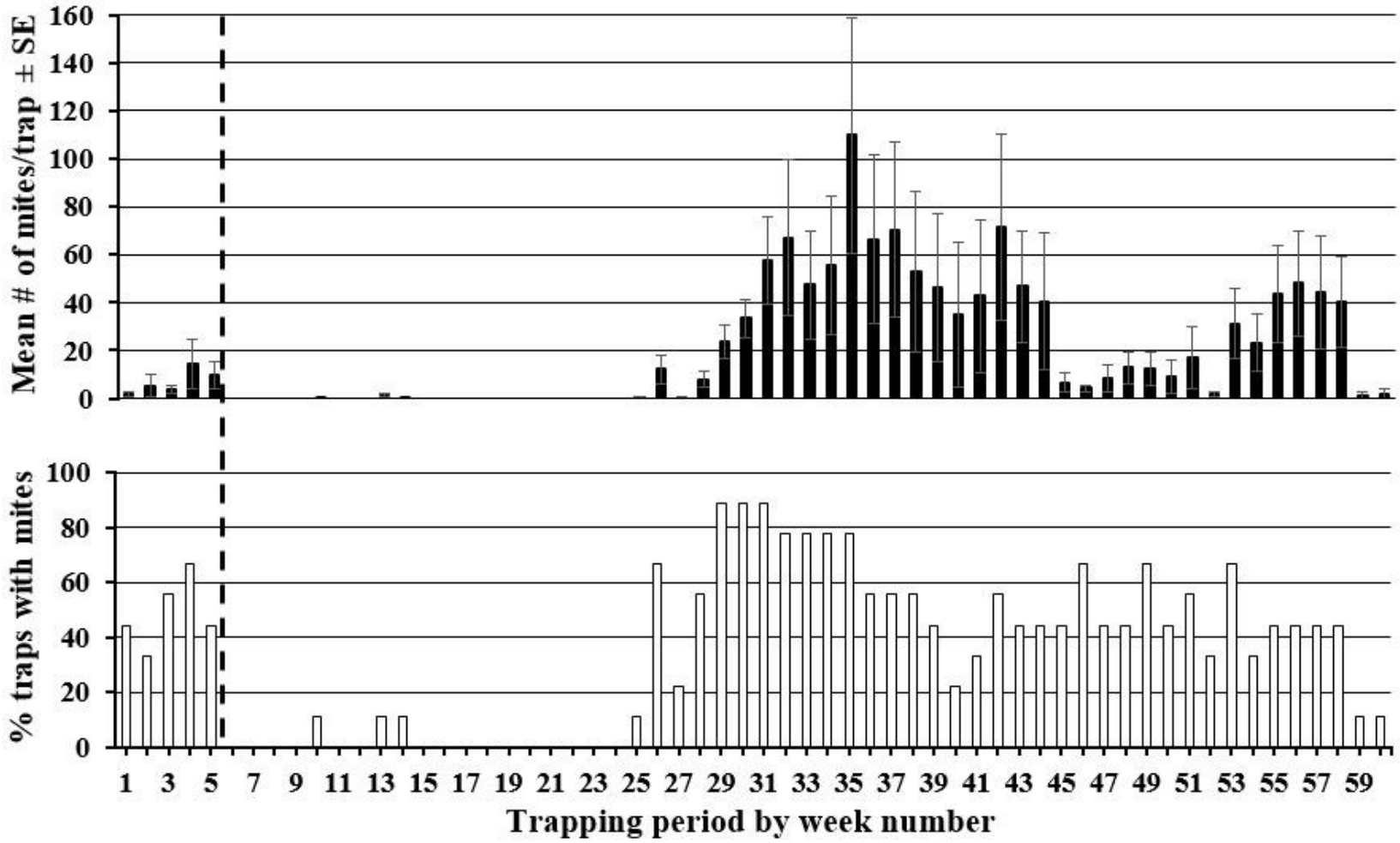

Figure 2.7 Illustration of the average weekly trap captures (above) and percentage of traps with *Tyrophagus putrescentiae* for Facility B, room 1.

The study was from June 2012 to September 2013. The dashed line indicates fumigation date.

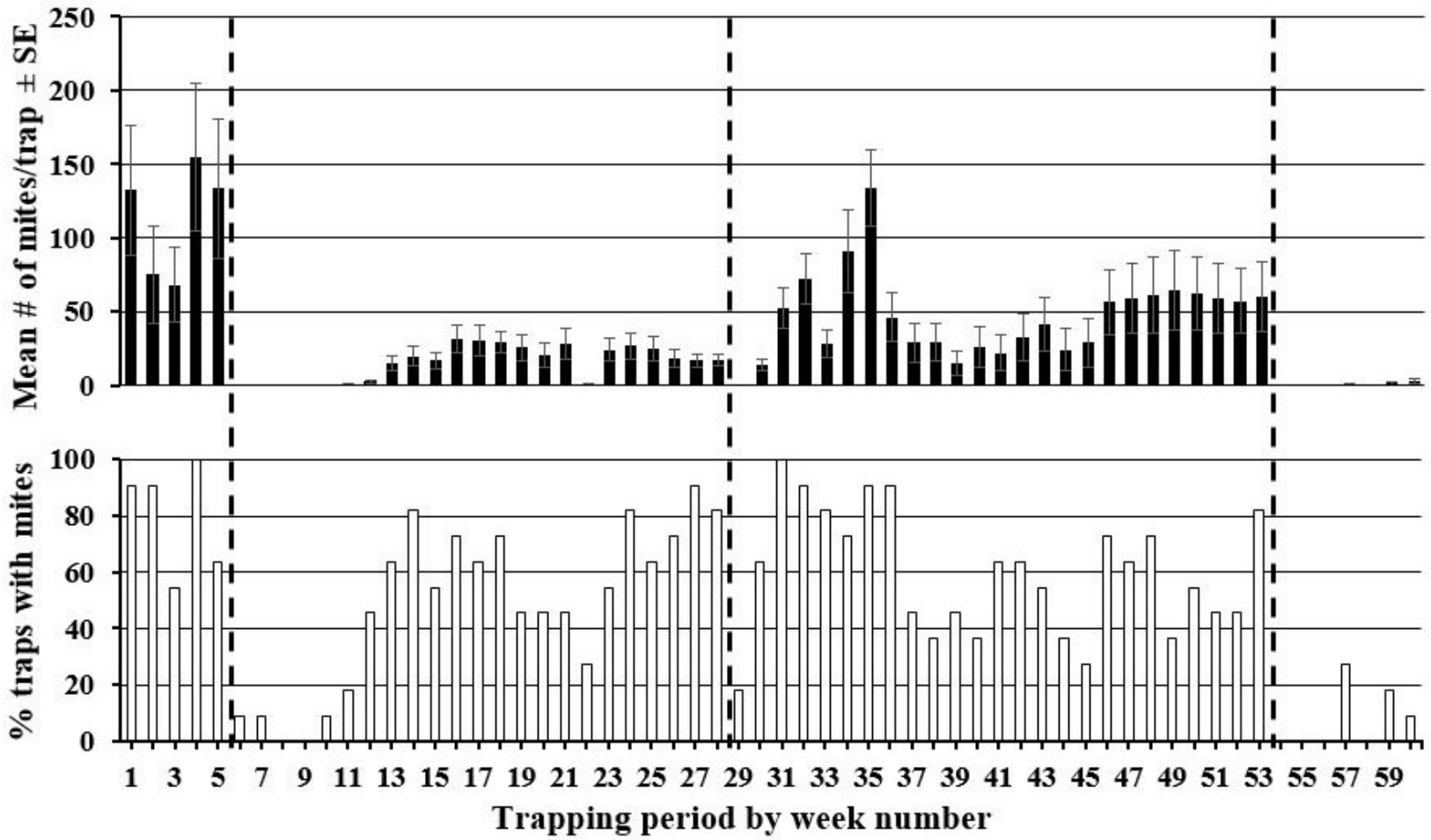

Figure 2.8 Illustration of the average weekly trap captures (above) and percentage of traps with *Tyrophagus putrescentiae* for Facility B room 2.

The study was from June 2012 to September 2013. The dashed lines indicate fumigation dates.

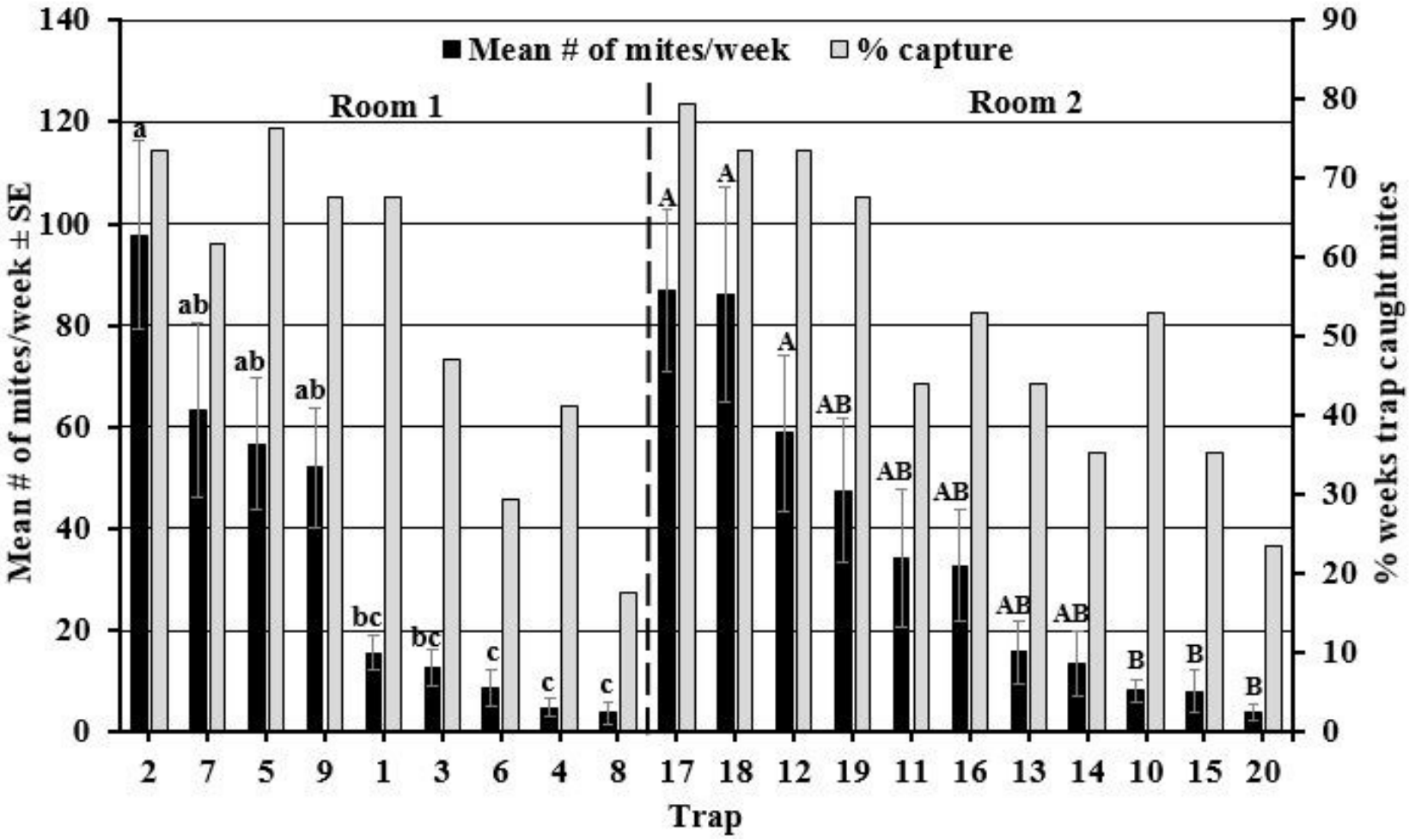

Figure 2.9 Trap ranking using mean (± SE) number of mites per week caught by each trap (left vertical axis) and percent trap capture (right vertical axis) in Facility B.

Trap ranking in two aging rooms (separated with dashed line) for 34 weeks from week 26 to week 59. Means with different letters are significantly different ($P < 0.05$; by Tukey-Kramer's Honestly Significant Difference Test).

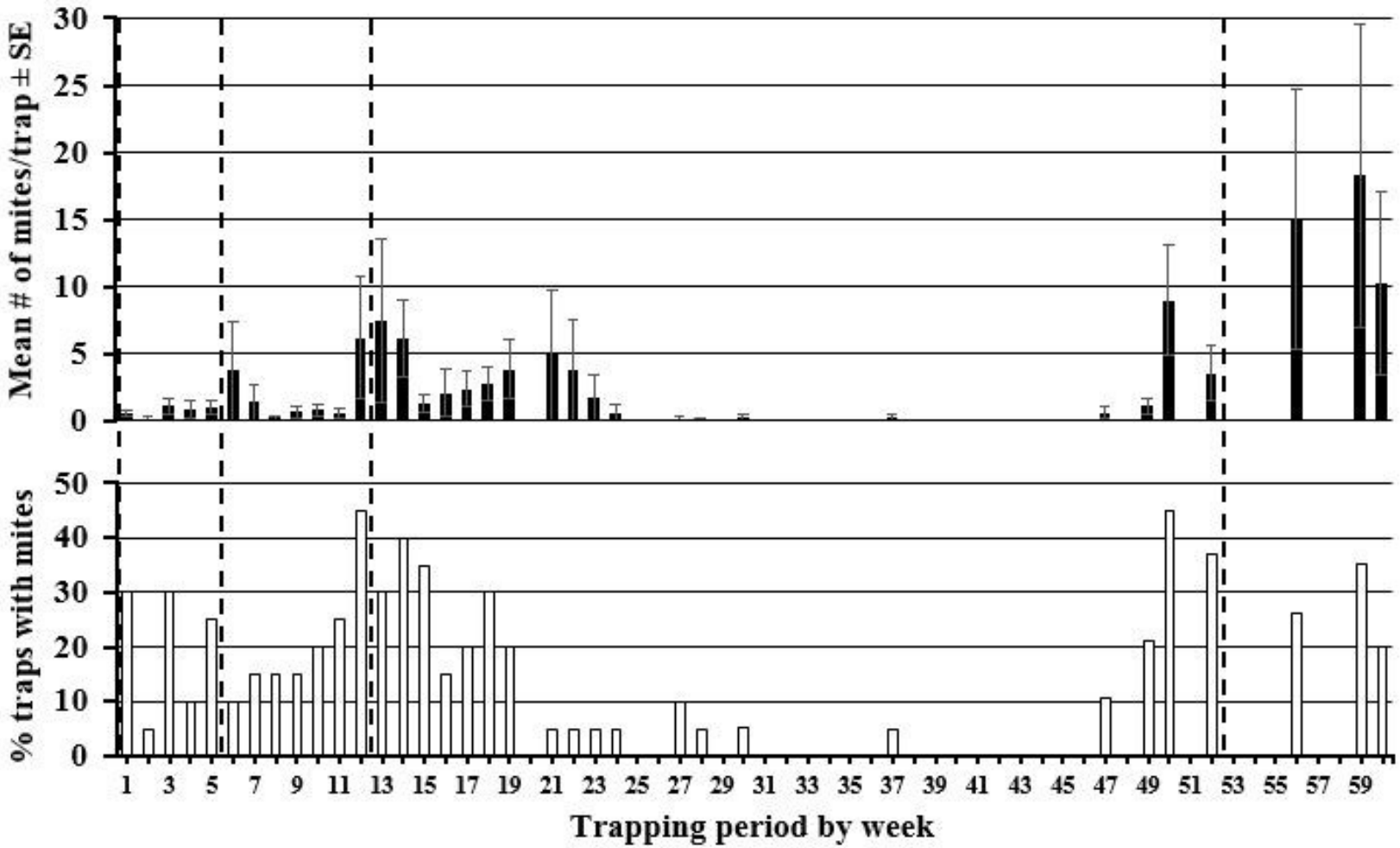

Figure 2.10 Illustration of the average weekly trap captures (above) and percentage traps with *Tyrophagus putrescentiae* for Facility C.

The study was from June 2012 to September 2013. The dashed lines indicate fumigation dates.

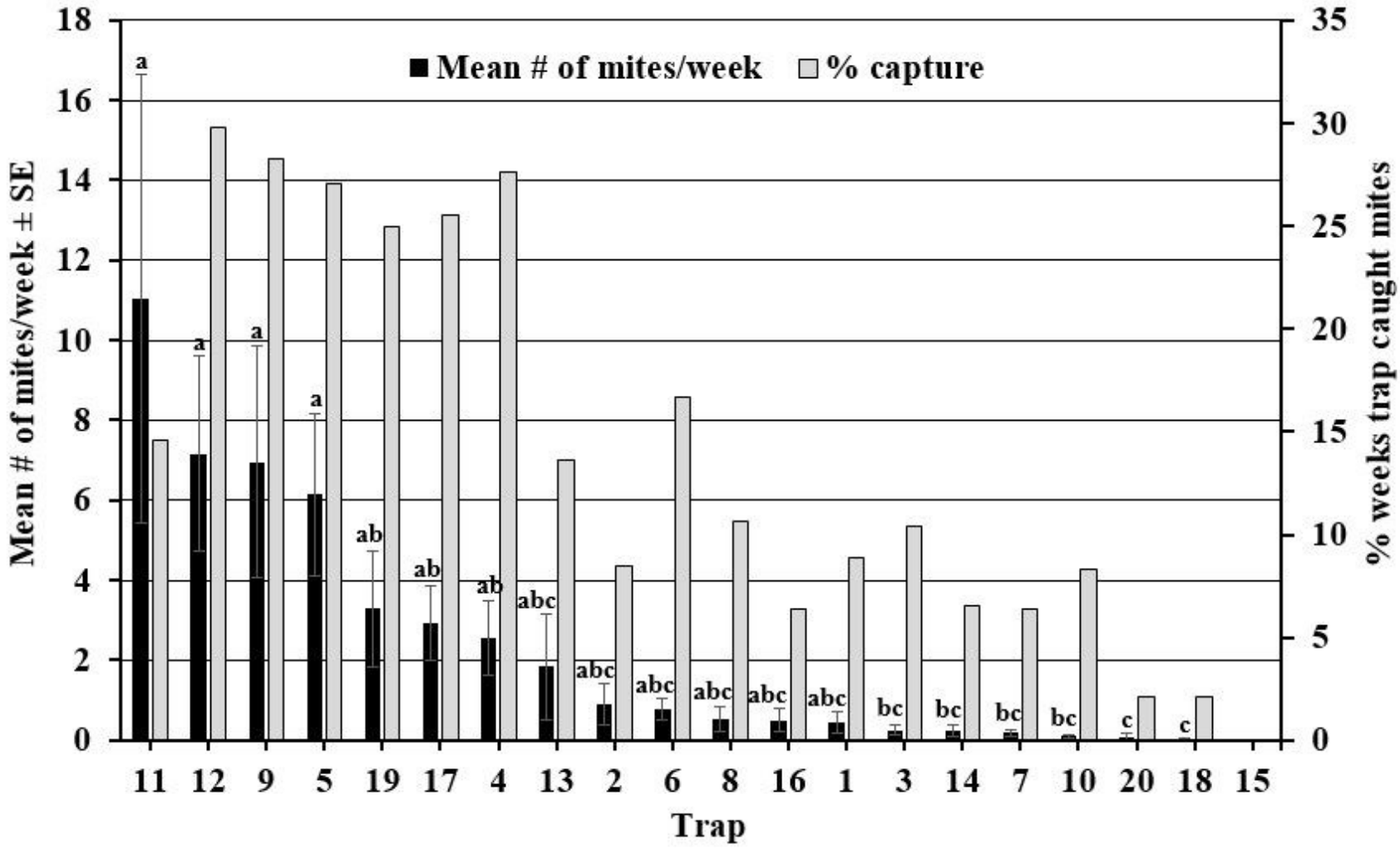

Figure 2.11 Trap ranking using mean (± SE) number of mites per week caught by each trap (left vertical axis) and percent trap capture (right vertical axis) in Facility C.

Trap ranking was for 60 weeks. Means with different letters are significantly different ($P < 0.05$; by Tukey-Kramer's Honestly Significantly different Test).

Chapter 3 - Physical factors influencing capture of *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) with food-baited traps

Abstract

The phase-out of methyl bromide, the most effective fumigant for the control of the ham mite, *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) on dry-cured ham, has resulted in the search for additional tools for integrated pest management. Early and effective detection of pests is useful in deciding if and when pest management is required. A standard trap made from disposable Petri dish and four commercial stored-product pest traps of different designs, were compared and evaluated for efficiency in mite detection. Additional experiments evaluated the effect of trap placement, trapping duration, and trap design that would maximize ham mite capture. A greater number of mites were captured in the standard trap when compared singly or side by side with commercial traps, so the standard trap was used in all other studies. Experiments in environmentally controlled rooms indicated that mites could be trapped up to six meters from a mite source within 24 hours. A greater number of mites were caught in traps placed along a wall than those away from a wall. Trap capture varied under different light conditions. More mites were caught in fully lighted rooms compared to those in fully dark rooms. However, an experiment in fully lighted rooms comparing unpainted (clear) traps with the standard black-painted traps found substantially more mites in black traps compared to the clear traps, suggesting that mites seek harborage from lighted conditions. Mites responded differently to light of different wavelengths. Light emitting diodes (LEDs) deployed with traps or assayed alone in small-scale orientation assays determined mites oriented positively to violet or ultraviolet, showed no preference to blue, and least preference with avoidance orientation to green, yellow, red and white lights. This research provides new information on orientation by *T. putrescentiae* and may help optimize trap designs or deployment for ham mites to help predict the population dynamics of the mite more accurately and provide good information for pest management decisions.

Introduction

Tyrophagus putrescentiae (Schrank) (Acari: Acaridae), commonly referred to as the mold mite, cheese mite, or ham mite, is a cosmopolitan, synanthropic post-harvest mite that has been reported to be associated with over 140 commodities (Hagstrum et al. 2013). *T. putrescentiae* has a high intrinsic rate of population increase (Sánchez-Ramos et al. 2007) and therefore poses considerable threat to valuable commodities that typically have high fat, protein and/or moisture contents. These commodities include pet food (Thind 2005, Brazis et al. 2008), cereal-based food products, dried fruits (Thind and Clarke 2001), cultured cheeses and dried meats such as southern dry-cured ham. Southern dry-cured ham becomes more susceptible to ham mite infestation when aged for more than five months, although aging for less than five months does not guarantee a mite-free ham (Rentfrow et al. 2006). Consumers prefer the unique taste and flavor of ham aged for longer periods, making the ham susceptible to mite infestation. The ham mite has been found to cause direct damage that reduces the quality and quantity of certain food products and this could result in product rejection (Jeong et al. 2005, Mueller 2010). In addition to being a serious pest of high-value foods, *T. putrescentiae* is a member of the house dust mite complex and has been reported to produce allergens and cause allergic reactions in humans (Jeong et al. 2005, Wakefield 2006).

The control of storage mites in food production premises can be difficult. Even with extensive hygiene measures, residual infestations of mites in hidden refuges may go undetected with the potential of subsequently infesting other products (Thind and Clarke 2001). The phase-out of methyl bromide fumigation, one of the most effective fumigants to control many stored-product pests including the mite, has led to the search for alternatives such as physical and biological methods, and use of other fumigants. The development of integrated pest management (IPM)

programs however still remains to be one of the most promising methods for stored-product pests (Fields and White 2002). The detection and monitoring of any pest is a critical aspect of every IPM program to ensure that pests are detected as early as possible, at population densities below the economic threshold. Informed decisions can therefore be made on management tactics with other alternatives to methyl bromide (Thind and Ford 2004).

Traps have been used since 2000 to detect and monitor storage mites that directly infest a commodity (Clarke 2002, Pacavira et al. 2006) and inside food and feed processing facilities (Thind and Ford 2004, Wakefield and Dunn 2005). However, very little research has been carried out with traps specifically designed for storage mites. The first commercially available mite trap, the BT® trap (Central Science Laboratory, York, UK), has been used in a number of detection and monitoring studies (Thind and Ford 2004, Thind 2005, Wakefield 2006). The BT® trap is a food-bait station constructed of 30% glass fiber that is reinforced with black nylon that has an 80 mm square base, and a 70 mm diameter lid that is attached to the base using a coarse screw thread and can be raised several mm to allow mites to enter, feed on the bait and remain or depart at their discretion. Thind (2005) carried out a study in four different types of premises: three of which were involved in the production of cereal based dry pet food, specialist animal feed, traditional cheese, and the fourth was the finished cereal section of a large transit storage warehouse. The trap detected low levels of at least seven species of mites including *T. putrescentiae*, *Acarus siro* (L.), and *Tyrophagus longior* (Schrank). This trap detected significantly more species of mites than the food bait bag composed of a mixture of cereal, peanut, and carob and a fishmeal trap, made of a moist filter paper with fishmeal covered with a raised Petri dish. Wakefield and Dunn (2005) studied the orientation behavior of three storage mites, *A. siro*, *T. longior*, and *Lepidoglyphus destructor* (Schrank) (Acari: Glycyphagidae), using the BT® trap and reported that the percentage

of mites found in the trap with bait was significantly greater than traps without bait. The authors also determined the distance that each species was able to reach a trap was approximately 80 cm within a 24 h period.

A simple trap was developed based on the general design of the BT® trap and used for detecting and monitoring the ham mite in commercial dry-cured ham facilities (Chapter 2, Amoah et al. 2016). Although that initial trap design was simple, inexpensive, and performed reliably, no in-depth work was done at that time to determine mite behavior associated with trap design and other variables that can affect trap performance. Many environmental factors have been studied in stored-product insects and were shown to increase trapping efficiency of those pests (e.g., Nansen et al. 2004, Duehl et al. 2011, Semeao et al. 2011, Toews and Nansen 2012). The objective of this research was to determine the effects of physical factors such as trap design, trapping duration, distance of trap from a mite source, trap location within a room, and lighting on trap capture and mite orientation to traps relative to light.

Materials and Methods

The standard trap and food bait

The standard trap for this study, the same trap used for chapter 2, was designed roughly according to the BT® trap. The standard trap was made of a plastic Petri dish with a 100 mm diameter that was 15 mm high, and spray-painted black on the outside. Eight evenly-spaced holes of approximately 0.5 mm diameter were made about 2 mm above the bottom along the side of the dish to allow entry of mites (Figure 3.1).

The food bait that was used in all traps for all experiments, unless indicated otherwise, was a 10 g circular disc that was 10 mm high with a 30 mm diameter. The diet for the food bait was made before each trial in batches of approximately 600 ml with the following ingredients: 475 ml water, 160 g dog food (a retail commercial product composed mainly of yellow corn, chicken by-product meal, beef, rice and soy), 40 ml glycerol (99.9%) (Fisher Scientific International, Inc.), 5 g brewer's yeast (MP Biomedicals, LLC), 10 g Agar (LCN Biomedicals, Inc.), 5 g alphacel (non-nutritive bulk cellulose) (LCN Biomedicals, Inc.), 5 g insect vitamin mix (MP Biomedicals, Inc.), and 5 ml of methyl-p-benzoate solution (15% methyl-p-benzoate in 95% ethanol) (LCN Biomedicals, Inc.). Details on the preparation of the bait are presented in Chapter 2.

Mite culture

T. putrescentiae for these experiments were from the laboratory culture at the Department of Entomology, Kansas State University, USA, which had been maintained since 2007. Mites were reared at 25 - 30 ^{0}C and 70 - 80% RH on a semi-moist dog food-based diet in 0.95 L Mason$^{(R)}$ jars (85 mm diameter, 160 mm height). The mixture used for the rearing diet had the same recipe, ingredients and quantities as the food bait described above except for the agar, which was halved

for the rearing diet. Approximately 100 ml of the finished hot mixture was added to each of six rearing jars that were half-filled with the same dog food as used in the hot liquid recipe and then mixed and allowed to cool. After cooling, approximately 10,000 mixed life stage mites were introduced into each jar. The metal jar lid inserts were replaced with filter paper (Whatman™/ cat No. 1001 090, 90 mm diameter) to ensure ventilation, and then held in place with the provided metal lid rings. Rearing jars were incubated for at least 14 days after which new mites could be harvested for use in experiments.

Experimental rooms

Trapping experiments were conducted in two identical small climate-controlled walk-in buildings that were each open spaces that measured 6 m long, 3 m wide and 2 m tall from the floor to the flat ceiling. Floors of each room were thoroughly cleaned at the end of each experimental replicate, at least 12 hours prior to the next replicate, by vacuuming the entire room and followed by mopping with water. The temperature was kept at 28 ± 2 ^{0}C and controlled with a thermostat and air conditioning/heating unit, while the relative humidity was maintained at approximately 70% using a humidifier. The temperature and RH were monitored once per hour with a data logger (HOBO U12-006 Temp/RH, Onset Computer Corporation, Pocasset, MA, USA) in each room to confirm that temperature and RH conditions were maintained throughout the duration of each experiment. For a given replicate, except when noted, a volume of new mites from a colony, equivalent to approximately 7200 adults with immature mites, were released at the mid-point of the floor wall junction along the 3 m wall opposite the door, once the experimental traps were deployed.

Effects of trap design

Four commercial traps (Figure 3.2), two of which are specifically designed for storage mites and two specifically designed for storage beetles, were compared with the standard trap in no-choice and two-choice experiments to determine if trap capture was affected by trap design. The two mite traps were the BT® trap and the Mite Monitor from Insects Limited Inc., Westfield, IN, USA. The two beetle traps were X-Lure multi species beetle floor trap from Russell IPM Ltd, Deeside, Flintshire, United Kingdom, and the Dome™ trap from Storgard® Trécé, Adair, OK, USA. The X-lure trap was slightly modified by replacing its snap-in pheromone/pitfall tray with a 100 mm diameter plastic petri dish in which the food bait was placed. In the no-choice tests, individual traps were placed 1.5 m from each side wall and 2 m from the mite source. Each of the six replicates for each trap design ran for 6 h in total darkness after which the number of mites in each trap was determined. The means were compared and separated on the basis of the Fisher's Protected Least Significant Difference test only when the F test of the ANOVA per treatment was significant at $P < 0.05$ (PROC GLM, SAS Institute 2010). In the two-choice experiments, the four commercial traps were each compared with the standard trap to determine the mite's preference, if any, for one trap design compared to the standard trap. A standard trap and a commercial trap were deployed at 2 m from the mite source and 2 m from each other. Traps were collected after 6 h and the number of mites in each trap were determined. Pairwise comparison of transformed proportion data (arcsine transformation) followed by a Two-Sample Student's t-test, assuming unequal variances was done ($P < 0.05$) (PROC TTEST, SAS Institute 2010).

Two food baits, the bait used in the standard trap (see above) and a commercial mite bait provided by Insects Limited Inc., were compared using standard traps in two-choice experiments. The Mite Monitor bait was a gelatinous material that reportedly contained a blend of plant and

animal products and chemicals that have attractive food odors and moisture for mites. The Mite Monitor bait mixture was prepared according to manufacturer's instructions and then dispensed into bait trays and refrigerated for at least two hours before use. The traps, one baited with the standard bait and the other baited with the Mite Monitor bait, were deployed at 2 m from the mite source and 2 m from each other in total darkness. Traps were collected after 6 h and the number of mites in each trap was recorded. In similar testing, a baited standard trap was compared with an unbaited standard trap in total darkness. Traps were collected after 6 h and the number of mites in each trap was recorded. For both experiments, pairwise comparison of transformed proportion data (arcsine transformation) was carried out, followed by a Two-Sample Student's t-test, assuming unequal variances *(P* < 0.05) (PROC TTEST, SAS Institute 2010).

The standard trap with 0.5 mm entrance holes was compared with variations that had either 2 mm, 4 mm, or 6 mm entrance holes. In each experiment, the standard trap and one of the three versions were deployed 2 m from the mite release point. The two traps were placed 2 m from each other and of equal distance from both long side walls. The room was completely dark. The traps were collected after 6 h and the number of mites in each trap was counted. Pairwise comparison of transformed proportion data (arcsine transformation) was carried out, followed by a Two-Sample Student's t-test, assuming unequal variances *(P* < 0.05) (PROC TTEST, SAS Institute 2010). All subsequent experiments were carried out using the standard trap.

Duration of trap deployment, trap distance from mite source, and trap location

The effect of trapping duration on mite capture was studied in a completely randomized design with five durations: 1 h, 3 h, 6 h, 12 h, and 24 h. A trap was placed 2 m from the mite source and 1.5 m from each side wall. Mites were released as before under total darkness and the

number in each trap were counted at the end of each trapping period. The effect of trap distance from mite source on mite capture was determined using four distances: 1 m, 2 m, 3 m, and 6 m. Each replicate was conducted for 24 h in total darkness after which mites in the trap were counted. Correlation analyses were carried out (PROC CORR, SAS Institute 2010) to determine if there were relationships between the duration of trap deployment, or trap distance from mite source, and the number of mites caught in the traps.

The effect of trap location on mite capture was studied in tests that examined horizontal and vertical traversal of mites. Traps were deployed 2 m from each other and 2 m from the mite source. One trap was placed at the edge of the long wall and the other 2 m away from and perpendicular to the wall. Traps were deployed for 6 h in total darkness after which the mites in the traps were counted. Pairwise comparison of arcsine-transformed proportion data was carried out, followed by a Two-Sample Student's t-test, assuming unequal variances *(P* < 0.05) (PROC TTEST, SAS Institute 2010). In two other experiments, the vertical movement of mites to traps placed on metal rack shelves above the floor was assessed. In one of these experiments, four traps were deployed, one each on shelves at 0.6 m, 1.2 m, and 1.8 m from the floor, with the fourth trap on the floor. Traps were deployed at one end of a shelf and the vertical orientation of the four traps formed a line perpendicular to the floor. Mites were released at the center of the topmost shelf, 1.8 m. In a similar experiment, mites were released on the floor underneath the rack and three traps were deployed, one on each of the three shelves. Both experiments ran for 24 hours. The means were compared and separated on the basis of the Fisher's Protected Least Significant Difference test only when the F test of the ANOVA was significant at $P < 0.05$ (PROC GLM, SAS Institute 2010).

Orientation of mites to light

The effect of room lighting on mite capture was studied in two experiments. A no-choice test was conducted in which a standard trap was deployed 2 m from the mite source and equidistant from both walls for 6 h in either total darkness or with the room light turned on. The light was provided by a 3780 lumen, 200 W crystal clear light bulb (General Electric Company, Nela Park, Cleveland, OH). A second experiment was designed to determine if mites avoided light in preference to dark places by comparing mite capture in a fully lit room in either a standard trap or an unpainted version of the standard trap in a series of two-choice tests. The traps were deployed 2 m from the mite source and 2 m from each other for 6 h, after which number of mites in each trap was recorded.

Light emitting diodes (LEDs) (RadioShack Corporation. Fort Worth, TX USA) generating light of different wavelengths and measuring 5 mm in diameter were used to determine the mite's preference, if any, for traps with an LED of specific wavelengths. Two-choice experiments were conducted in the dark with paired standard traps in which one trap had no LED and the second was equipped with one of seven LEDs fixed on top of the trap, pointing toward the mite source. The LEDs tested and their manufacturer-reported wavelengths were red (660 nm), yellow (585 nm), green (570 nm), blue (468 nm), violet (460 nm), ultraviolet, UV (405 nm), and white (400-800 nm). The power supply for each LED was a series of 2 – 4 AA 1.5V batteries (Duracell Berkshire Corporate Park, Bethel, CT). The two traps were deployed 2 m from each other and at 2 m from the mite source for 6 h. Pairwise comparison of transformed proportion data (arcsine transformation) followed by a Two-Sample Student's t-test, assuming unequal variances ($P < 0.05$) (PROC TTEST, SAS Institute 2010) was done. An 8-choice experiment was also conducted with eight standard traps in which seven of the traps were each equipped with a different LED as

described above, and the 8th trap had no LED. The traps were deployed for 6 h in a dark room in a circle at different distances from each other and at 1 m from the mite source, which was at the center of the floor. The position of a trap was randomized for each replicate. The means were compared and separated on the basis of the Fisher's Protected Least Significant Difference test only when the F test of the ANOVA was significant at $P < 0.05$ (PROC GLM, SAS Institute 2010).

Another set of experiments was conducted to determine the orientation preference of the mites to LEDs alone without traps or bait. The observation arena was a 10 cm dia circle drawn with pencil on a rectangular sheet of black construction paper. A new piece of construction paper was used for each replicate. The arena was then divided into 10 equally-spaced 1-cm regions by drawing nine parallel lines perpendicular to the long dimension of the paper. The central line, which was 5 cm from either end of the arena, ran across the diameter of the arena. An LED for testing was placed at one end of the arena with its beam shining perpendicular to the spacing lines, and a group of 10 mites was released at the center of the circular arena. Mites were observed for a 300 seconds in a dark room (except for illumination from the LED being tested). The time taken for a mite to reach within 1 cm of the light source was recorded. Since observation was ended at 300 seconds, the data were treated as right-censoring data (Klein and Moeschberger 2003). This analysis accounts for mites that could reach the goal after 300 seconds. It was assumed that the data followed a log-normal distribution and therefore fitted a linear model. An estimate time was then generated for traversal time to each light and all pairwise comparisons were made to find differences between any two lights. The means were compared and separated on the basis of the Fisher's Protected Least Significant Difference test only when the F test of the ANOVA was significant at $P < 0.05$ (PROC LIFEREG, SAS Institute 2010).

Results

Effects of trap design

In the no-choice experiments, there was no significant differences between the standard trap and the Mite Monitor, which both caught more than the other traps tested (Figure 3.3). The standard trap caught significantly more mites than any of the four commercial traps in separate two-choice experiments (Figure 3.4). Two-choice experiments that compared the standard bait with the Mite Monitor bait found no differences in the number of mites caught ($P = 0.14$) with 113.2 ± 4.1 mites in the traps with the Mite Monitor bait and 105 ± 3.9 in traps with the standard bait. The presence of a bait in the standard trap was critical for trapping mites as an average of 101.3 ± 6.2 was recorded in baited traps compared to only 7.0 ± 1.7 in unbaited traps ($P < 0.01$).

Two-choice experiments found that hole size did not affect the proportion of mites trapped as 44.5 ± 1.9% were in traps with 2 mm holes, 44.3 ± 3.0% in traps with 4 mm holes, and 42.6 ± 3.6% in traps with 6 mm holes compared with those in the standard trap with 0.5 mm holes.

Duration of trap deployment, trap distance from mite source, and trap location

Mite capture in traps at 2 m from the source was positively correlated with duration of trapping (Figure 3.5). Studies on the trap distance from mite source showed a strong negative correlation between trap distance from mite source and the number of mites caught in traps (Figure 3.6) indicating that the closer the trap is to the mite source, the higher the number of mites caught. Mites captured in traps placed along the wall (197.0 ± 12.1) were almost three times more than in traps placed away from the wall (74.2 ± 7.4) in two-choice experiments ($t_{10} = 18.44$, $P < 0.01$). There were significant differences between traps placed at different heights. When mites were released on the topmost shelf, the most mites, 309.3 ± 16.8 mites per trap, were captured in the

trap placed on the floor and the least number of 146.8 ± 7.2 mites per trap was captured in traps placed on the shelf close to the mite release point that was 1.8 m from the floor. An average of 185.7 ± 12.3 mites and 184.0 ± 7.1 mites were caught in traps placed on the shelves that were 0.6 m and 1.2 m from the floor respectively and these were not significantly different from each other but significantly different from trap capture on the floor and on the topmost shelf ($F_{2, 15} = 258.08$, $P < 0.01$). When mites were released on the floor, 58.8 ± 7.0 were captured in the trap on the shelf 0.6 m from the floor and 11.3 ± 1.5 mites and 0.8 ± 0.5 mites were captured in traps placed on the shelves that were 1.2 m and 1.8 m respectively from the floor ($F_{3, 20} = 37.42$, $P < 0.01$).

Orientation of mites to light

Fewer mites, 105.5 ± 3.8, were caught in standard traps deployed in completely dark rooms than in fully-lit rooms where an average of 211.3 ± 10.0 mites were captured ($P < 0.01$). When the unpainted trap and the standard trap were compared in lit rooms, the standard trap caught 152.7 ± 7.7 mites, which were more than the 71.2 ± 4.9 mites that were captured in the unpainted trap ($t_{10} = 11.46$, $P < 0.01$).

In the two-choice experiments in which one standard trap had an LED and the other was unlit, mites oriented more positively to traps with violet and UV LEDs. Traps with blue LED caught similar numbers of mites when compared to unlit traps, while traps with yellow, green, red, or white LED consistently had fewer mites in comparison to unlit traps (Figure 3.7). In the 8-choice experiments with all seven LEDs and an unlit control, traps with UV or violet LED caught the highest numbers of mites ($P < 0.05$) in comparison to all other light treatments. (Figure 3.8).

Mites oriented to the UV and violet LEDs in shorter times than to other LEDs. Red was the least preferred. (Figure 3.9).

Discussion

The results of this study provide important information on selected factors and their effects on trap capture of *T. putrescentiae*. The experiments confirm that the simple standard trap for *T. putrescentiae*, composed of a black-painted disposable Petri dish with small holes near the floor for mite access to a food bait, can be useful and versatile for monitoring mite populations in dry-cured ham aging rooms (Chapter 2). Experiments with various commercial trap designs demonstrate the importance of the mite-sized holes for retaining mites that have oriented to the food bait, as both the standard trap and the Mite Monitor had the highest mite numbers. In comparison, a shared feature of the BT®, Dome, and X-lure traps, relative to the standard trap and the Mite Monitor, is that they each have much larger openings for mites around the base of the traps. The hole size experiment with the standard trap demonstrated that fewer mites were trapped with larger holes, so it is therefore important that traps with smaller openings are used for greater sensitivity to mite activity. These results infer that the standard mite trap, or similar designs with minimal access for mite entry, is good for mite harborage perhaps due to safety from larger intruders or fewer opportunities to leave once inside. Storage mites prefer to hide in cracks and crevices with food in walls or in other indoor structures (Thind 2005, Wakefield and Dunn 2005). *T. putrescentiae* may therefore be like many stored-product insect pests, which prefer a less open or more restricted habitat once appropriate food is encountered (as in Epsky et al. 2008) which could be provided best by traps with small holes.

Trapping duration and trap placement experiments suggested that the standard mite trap was very effective at detecting mites that travel a relatively long distance within one day of dispersing from a spent food or harborage site into a relatively food-free area. (e.g. Amoah et al. 2016, Chapter 2). Food-baited traps that detect pests within the shortest possible time are preferred

in pest management programs since such traps are likely to otherwise become a source of re-infestation if left deployed for a long time (Collins and Chambers 2003, Hagstrum and Subramanyam 2006). The present work suggests that trap encounter by mites over space and time in complete darkness is simply a sampling of mites from huge numbers that are dispersing evenly in an expanding radius from the starting point. The highest numbers of mites are sampled close to the source, while numbers in traps decrease with distance from the source as mites disperse and spread out to lower densities. Mites that encounter a wall are likely concentrated in numbers since the wall directs their path; approximately three times more mites were found in traps along the side wall of the rooms compared to traps place in the center of the rooms. Regardless of mite release along a vertical transect of the space, whether from the floor or from a higher aspect, *T. putrescentiae* seems to be positively geotactic. This mite species, like many other stored-product pests and mobile insects in general, may be using the walls and vertical structures like the rack legs in this case, as guides for ambulation leading them to traps along walls and on the floor. Studies with stored-product insects have shown that traps in undisturbed locations at floor corners and along walls capture more insects than those in the open (e.g., Toews and Nansen 2012 for beetles).

This work demonstrated that light plays important roles in the orientation and activity of *T. putrescentiae*. When traps were in fully lit rooms there were many more mites in the standard trap compared to the unpainted trap, suggesting that mites seek refuge from lit spaces. This aversion to light by *T. putrescentiae* is not unlike similar behavior relative to traps for several species of stored-product insects (Toews and Nansen 2012) and validates the placement of mite traps in darkened, protected areas for best chances of detection in pest management programs. The studies on orientation of *T. putrescentiae* relative to wavelength of light indicated that mites moved towards violet and ultra-violet light and moved away from red, yellow, green and white light,

either when combined with a food-baited trap or as a stand-alone stimulus. This suggests that the ham mite, like many other mites and insects, responds differently to different light wavelengths. Furumizo (1975) indicated that acarine mites may possess photoreceptors since the house dust mite, *Dermatophagoides farinae* (Acari: Pyroglyphidae) responds positively to green (500 - 575 nm) and negatively to orange/red (600 - 675 nm). *D. farinae* does not respond to violet/blue (350 - 475 nm) unlike the ham mite that showed a great preference to violet in the current study, with response to blue being neutral. Other mite studies have shown that adults of the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae) and some predatory mites would generally avoid UV (Barcelo 1981, Onzo et al. 2010, Sakai and Osakabe 2010, Suzuki et al. 2013). However non-diapausing females of *T. urticae* are attracted to green, white, blue and red whereas diapausing females show no preference for visible radiation (Suzuki et al. 2013), suggesting that *T. urticae* has at least two types of photoreceptors (Suski and Naegele 1963, McEnroe and Dronka 1969, Mills 1974). Wavelengths shorter than the UV studied here, such as UV-B (280-315 nm) and UV-C (200-280 nm), have however been reported to be lethal to *T. urticae* (Barcelo 1981) and *T. putrescentiae* (Bakr 2013) and thus are not likely stimuli for positive orientation. Many stored-product insects are attracted to UV-A light (315 - 400 nm) and therefore many light traps utilize UV light as an attractant (Nualvatna et al. 2003, Epsky et al. 2008, Duehl et al. 2011). Other studies have shown that many insects possess green light sensitive receptors and therefore the use of green LED alone or in combination with UV LED could result in higher trap capture (Epsky et al. 2008, Stukenberg et al. 2015). The preference of some wavelengths by *T. putrescentiae* in the current study, and by other arthropods as cited here, may help explain that arthropods develop and utilize different photoreceptors sensitive to a variety of wavelengths depending on factors such as their ecological habitat, behavior relative to their habitats, and physiological state for survival.

Responses obtained in the current study also suggest that a long-distance stimulant, such as the violet or UV light could aid in bringing individual ham mites close enough to a trap to be attracted by the food bait and this would increase trap catch. A more specific adaptive reason underlying the positive orientation by *T. putrescentiae* to shorter wavelengths such as the UV is not readily apparent, but it is possibly an adaptation to life in a low light environment such that certain targets for orientation are more easily detected than in full light conditions.

The performance of traps for *T. putrescentiae* may be improved for pest management with behavioral cues including visual stimuli such as color, light, and shape or design, and chemical lures such as those related to food/host, habitat and pheromones. Such cues help make traps more specific and/or effective (Epsky et al. 2008). The current work provides information that may help increase trapping efficiency for *T. putrescentiae* and in the development of better trapping and monitoring methods for this pest. Deployment of the standard trap tested here in food facilities can be optimized with floor placement in dark conditions and along walls or similar structural edges. Results from work with light suggests that trapping could be enhanced with UV light at a trap, and that reduced mite activity on exposed food (e.g., aging hams) or food storage buildings in general can be achieved by full continual lighting with white or red lights. Up to now, there has been only limited work on integrated pest management programs for *T. putrescentiae*, but recent studies focused on IPM solutions for the loss of methyl bromide fumigation (e.g., Abbar et al. 2016a, 2016b; Amoah et al. 2016, Zhao et al. 2016) suggest that an effective trap for detection and monitoring of this pest will be important for its IPM moving forward.

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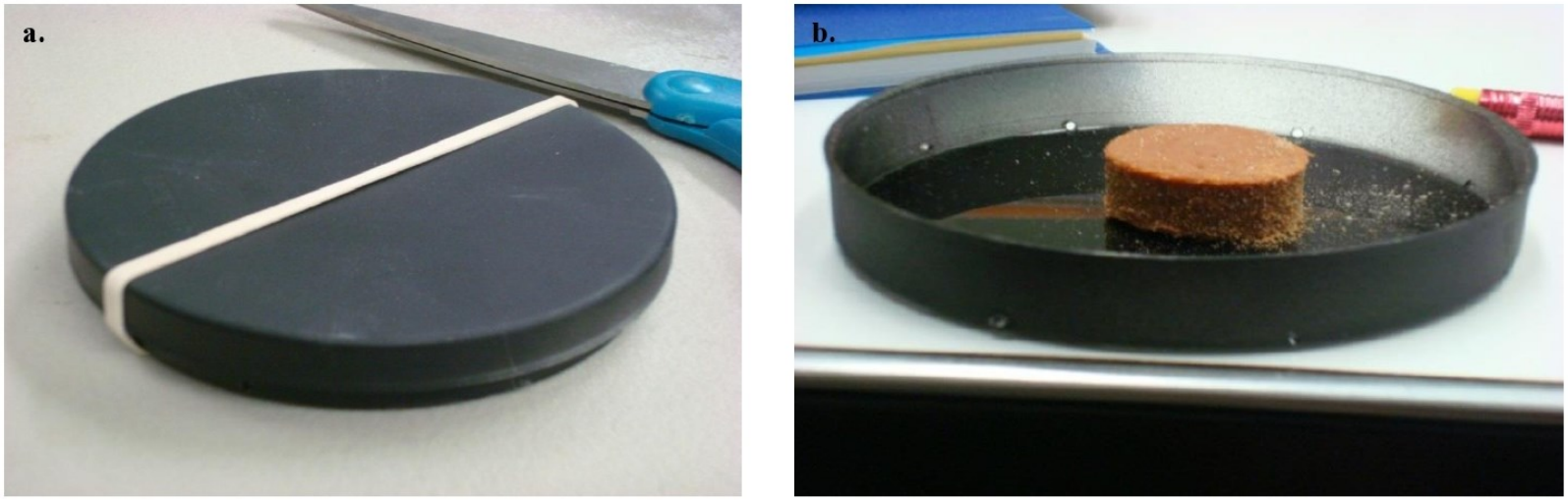

Figure 3.1 Standard mite trap closed (left), and opened (right), revealing food bait and entrance holes.

The trap was made from a black-painted disposable 100 x 15 mm Petri dish.

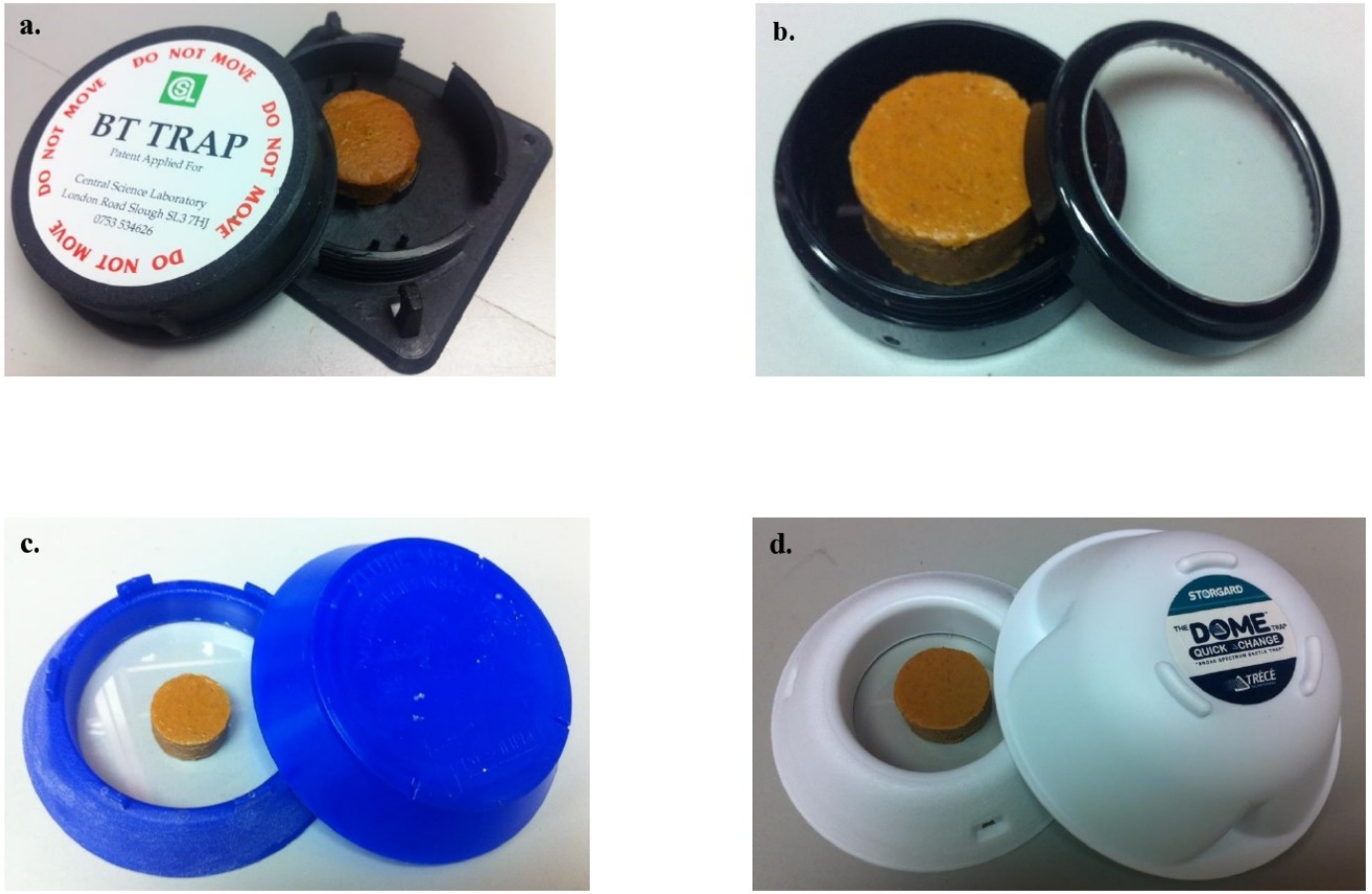

Figure 3.2 Commercial traps used in trap studies.

(a) BT® mite trap. (b) Mite Monitor (c) X-lure multi species beetle trap (d) Dome trap. All traps were baited with the standard food bait unless indicated otherwise. The food bait shown in each photograph is 30 mm diameter, which can assist in judging the relative sizes of these traps.

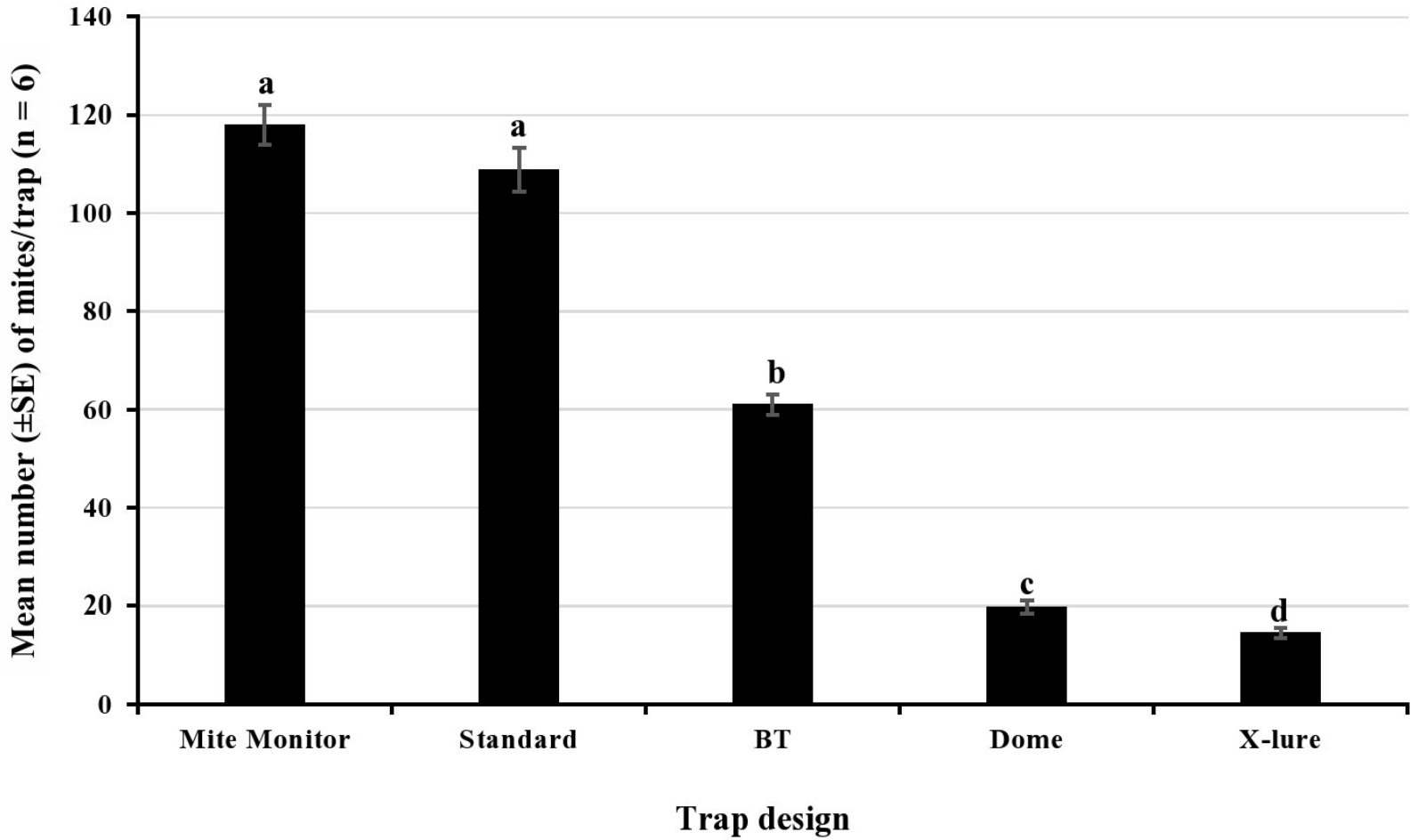

Figure 3.3 Response of *T. putrescentiae* to individual traps of different designs in no-choice experiments.

The means were compared and separated on the basis of the Fisher's Protected Least Significant Difference test only when the F test of the ANOVA per treatment was significant at $P < 0.05$ (PROC GLM, SAS Institute 2010). Means with different letters are significantly different ($F_{4, 25} = 270.70$, $P < 0.01$).

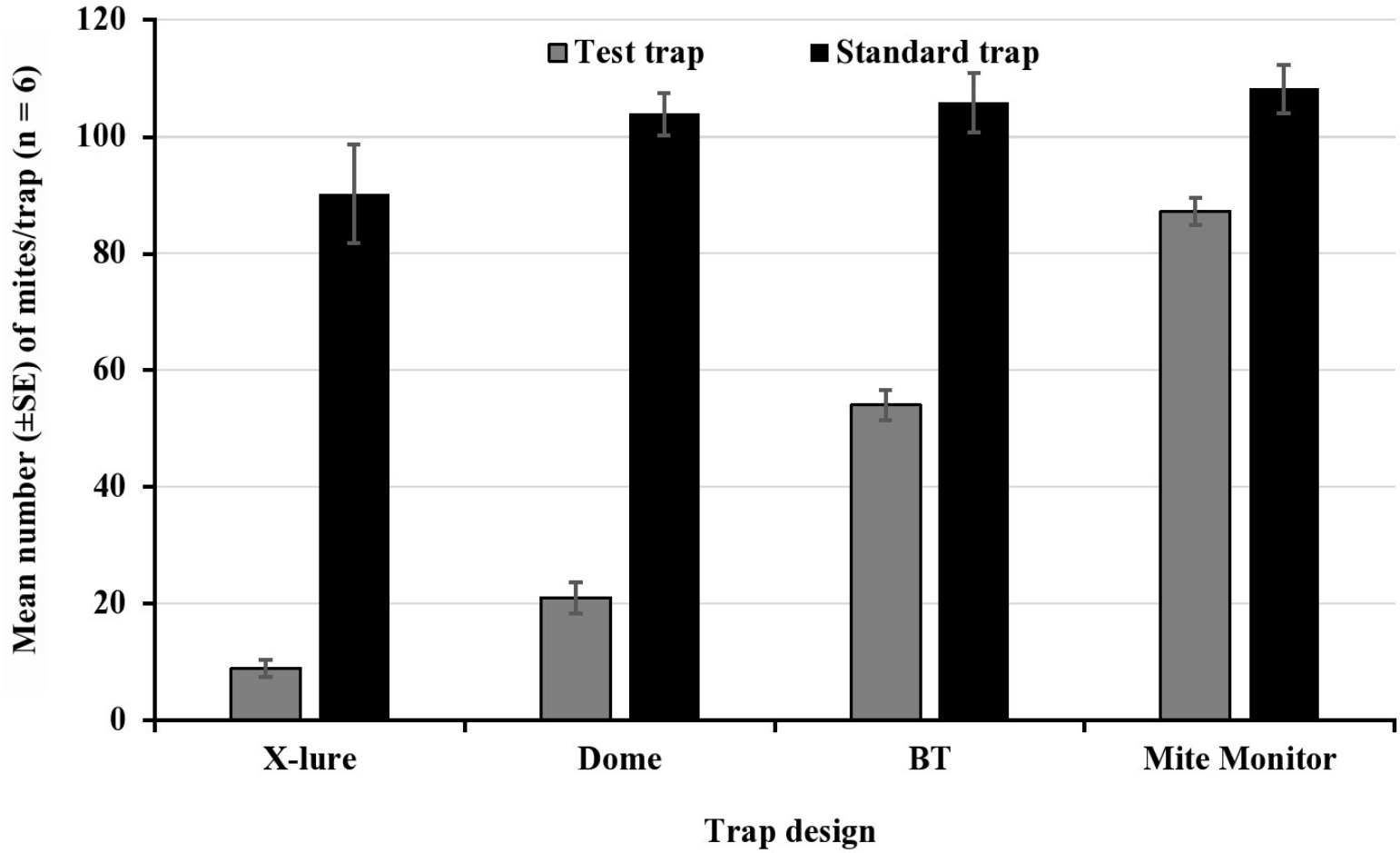

Figure 3.4 Effect of trap design on trap capture of *T. putrescentiae* in two-choice experiments.

Pairwise comparison of transformed proportion data (arcsine transformation) followed by Two-Sample Student's t-test, assuming unequal variances. All pairwise comparisons were significantly different at $P < 0.01$. (PROC TTEST, SAS Institute 2010).

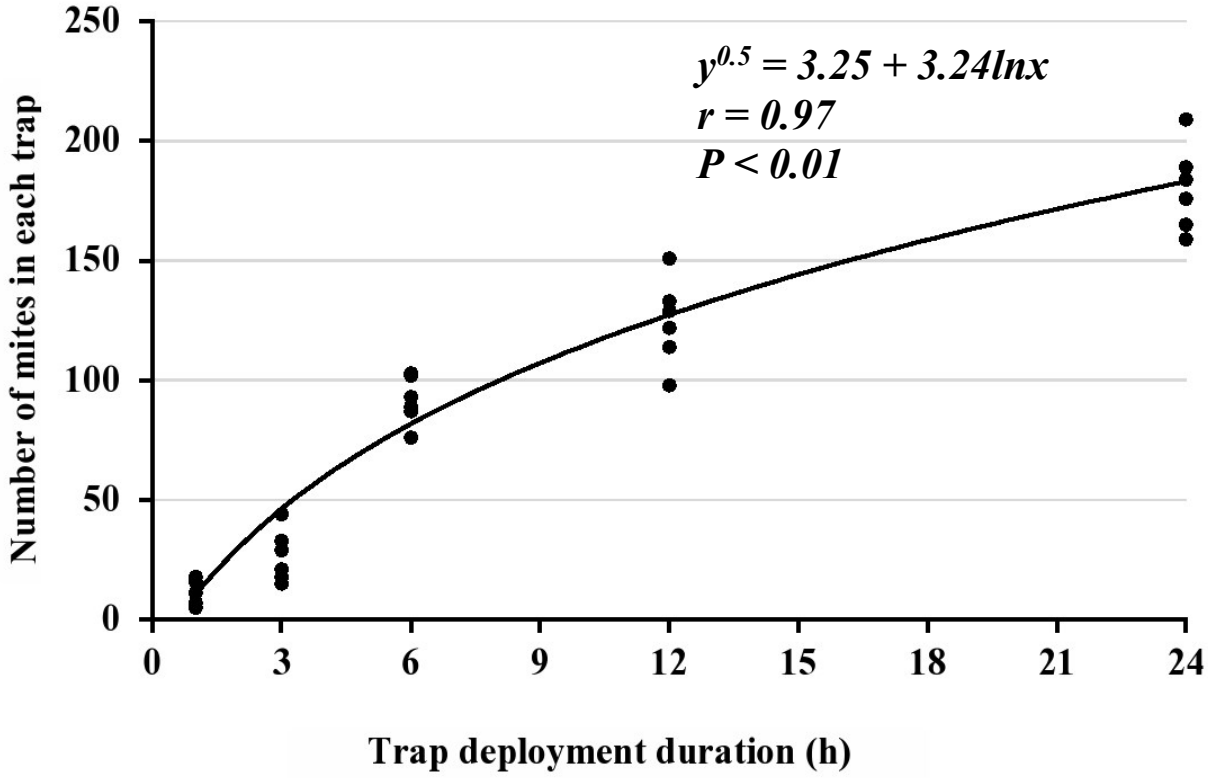

Figure 3.5 Effect of trap deployment duration on trap capture of *T. putrescentiae* in no-choice experiments.

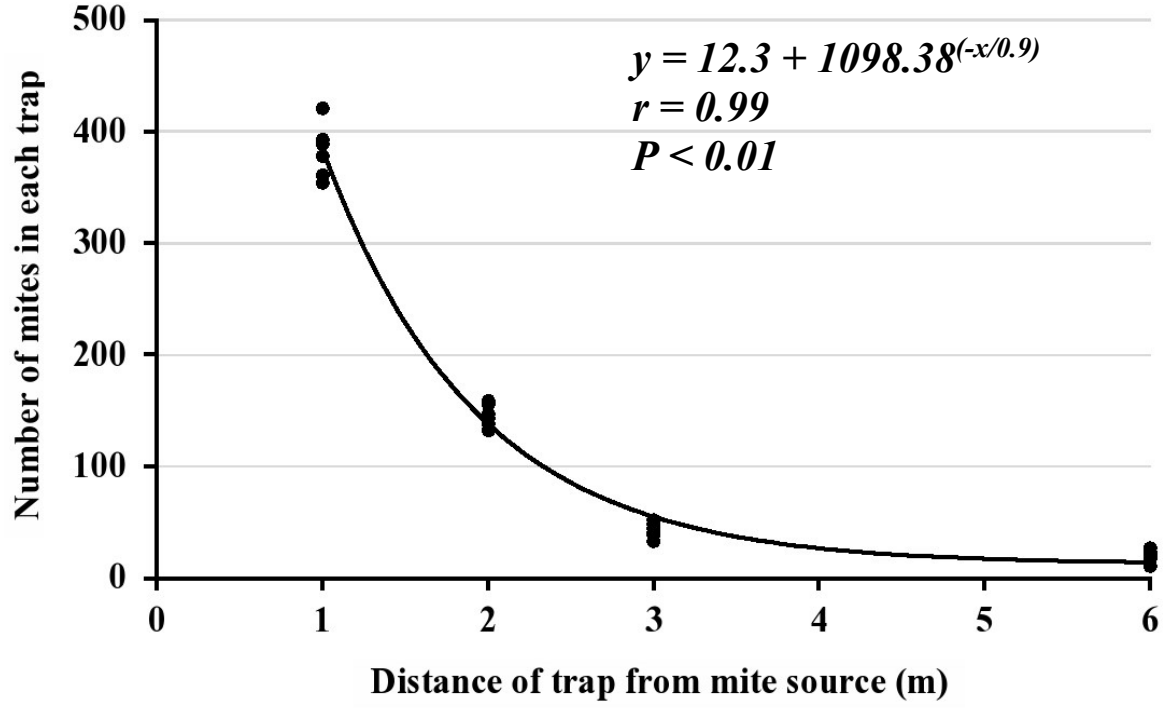

Figure 3.6 Effect of trap distance on capture of *T. putrescentiae* in no-choice experiments.

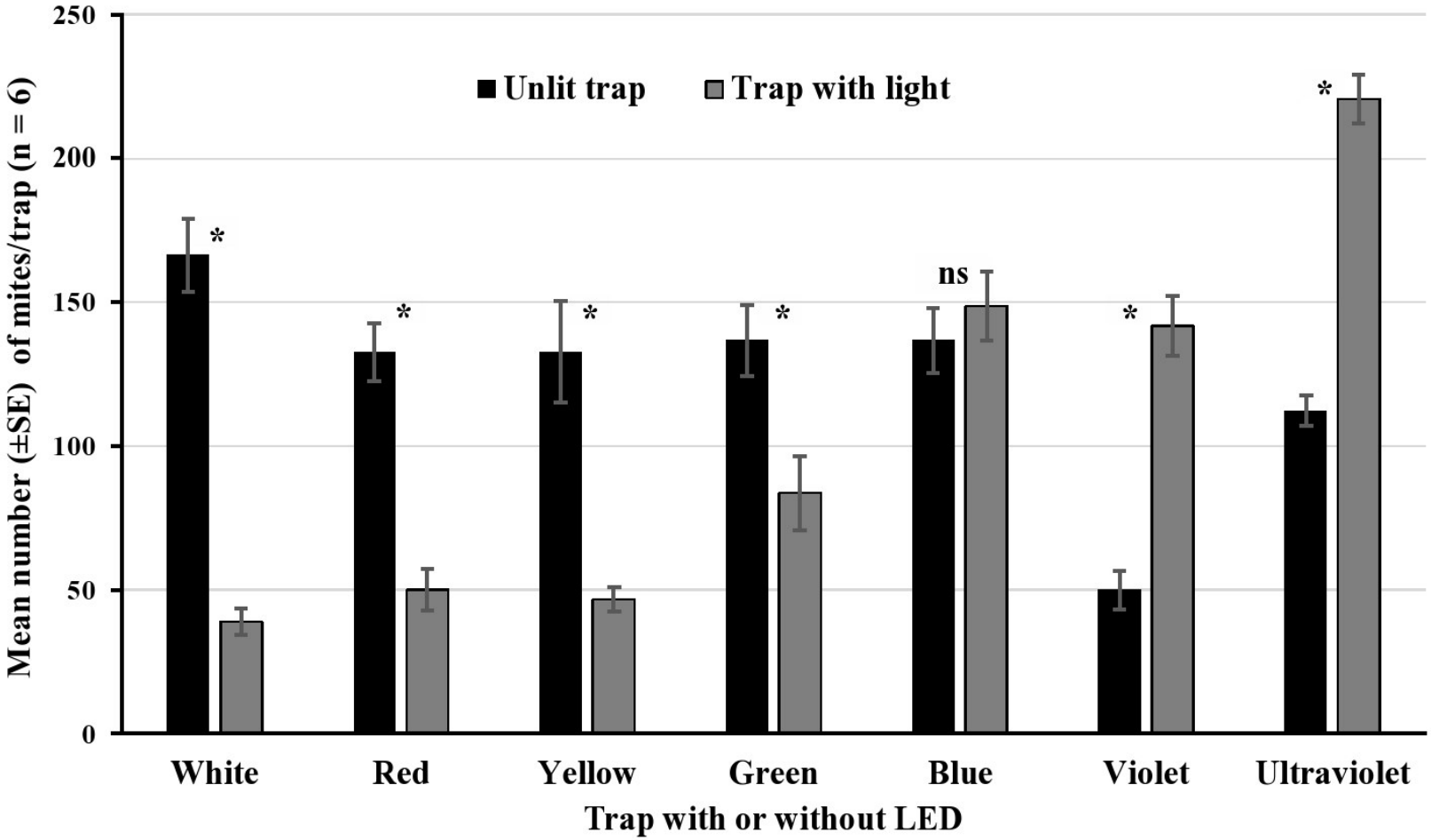

Figure 3.7 Capture of *T. putrescentiae* in traps with or without Light Emitting Diodes in two-choice experiments.

Pairwise comparison of transformed proportion data (arcsine transformation) followed by Two-Sample Student's t-test, assuming unequal variances. (ns = not significant; * = $P < 0.01$) (PROC TTEST, SAS Institute 2010).

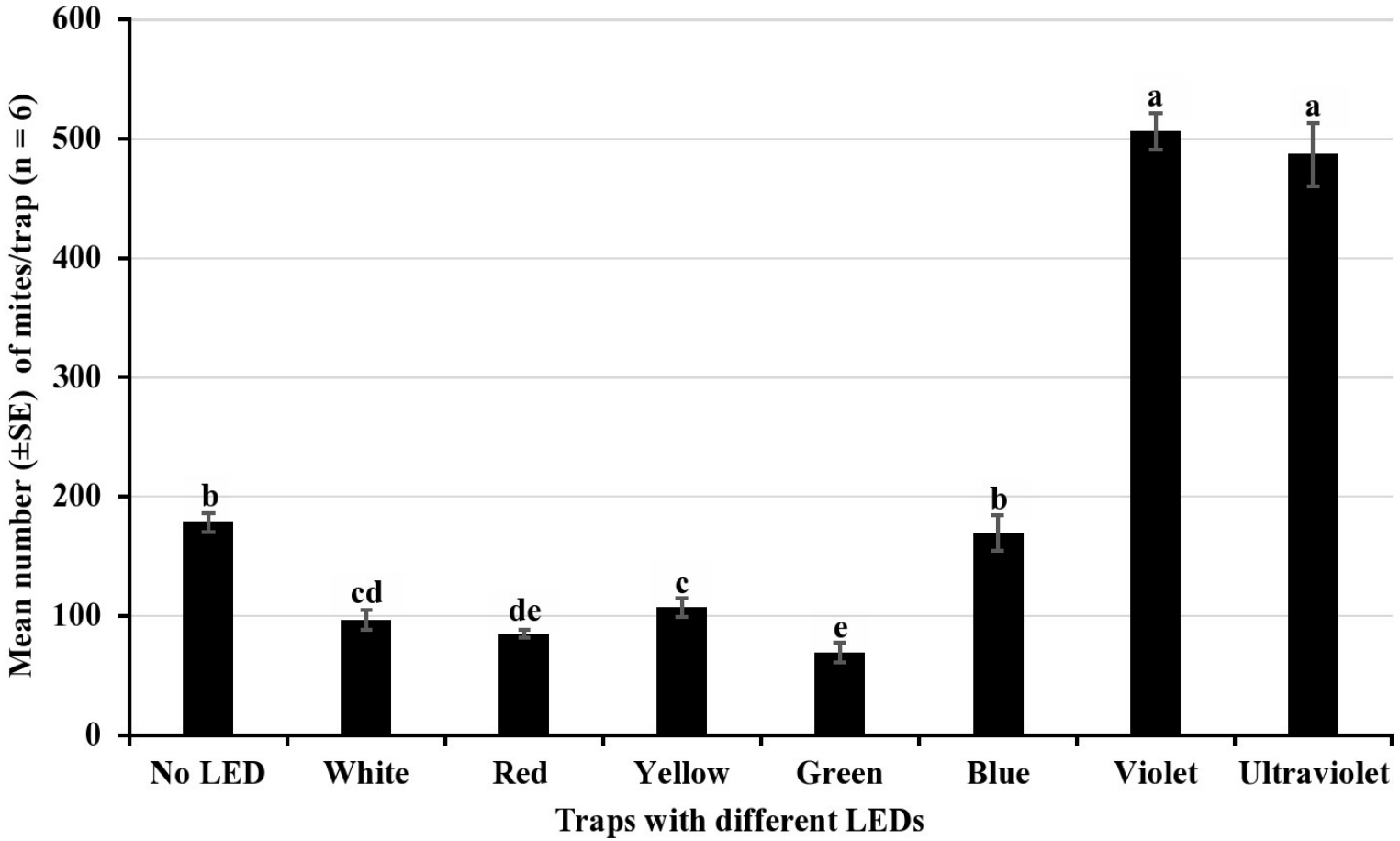

Figure 3.8 Capture of *T. putrescentiae* in traps equipped with light emitting diodes in an eight-choice experiment.

The means were compared and separated on the basis of the Fisher's Protected Least Significant Difference test only when the F test of the ANOVA per treatment was significant at $P < 0.05$ (PROC GLM, SAS Institute 2010). Means with different letters are significantly different ($F_{7, 40} = 248.89$, $P < 0.01$).

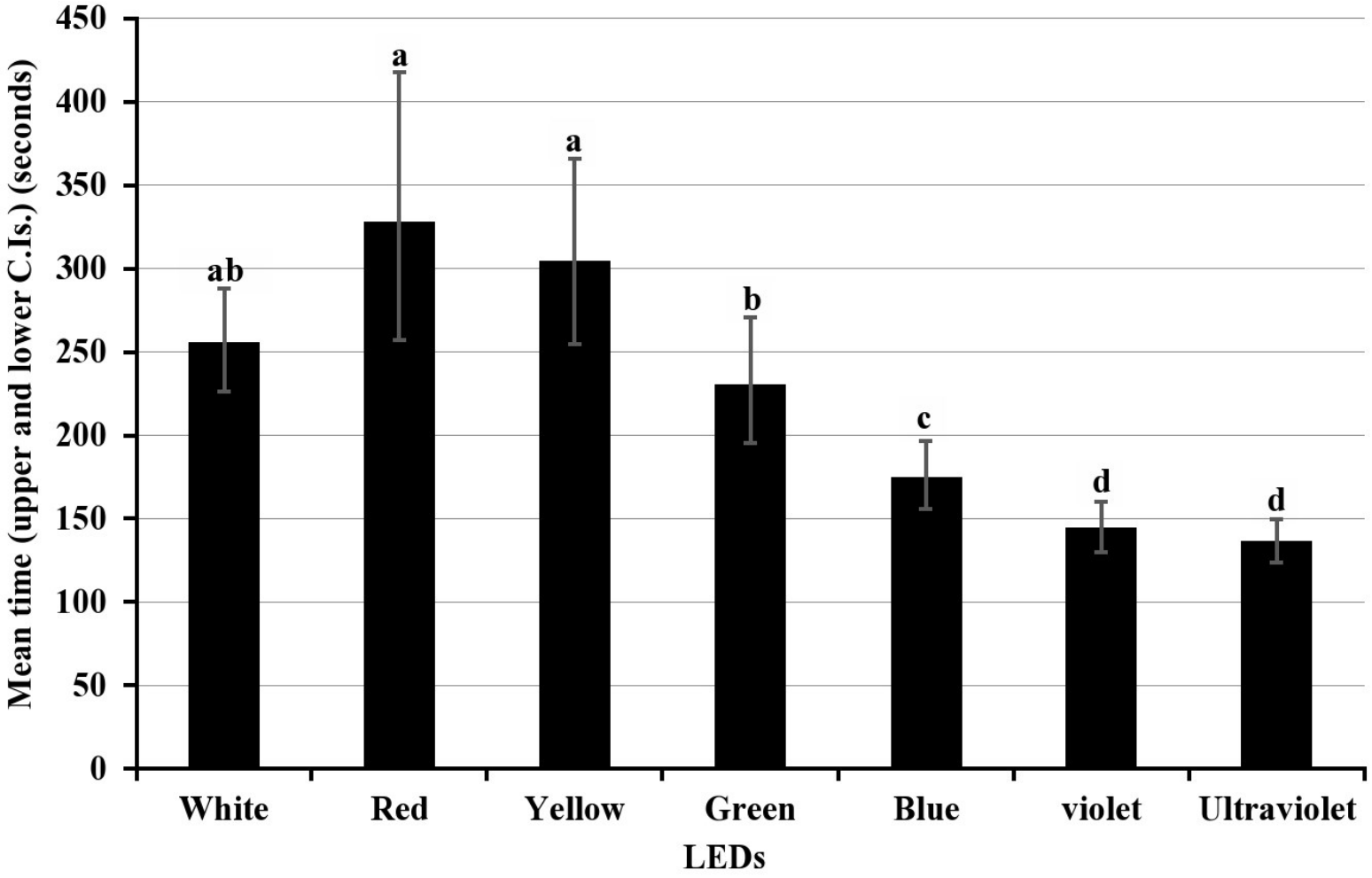

Figure 3.9 Mean orientation time (upper and lower confidence intervals) in seconds for individual *T. putrescentiae* to reach within 1 cm of a light emitting diode of a given wavelength.

Experiment was carried out using a 10 cm walking assay. Means with different letters are significantly different ($P < 0.05$) (PROC LIFEREG, SAS Institute 2010).

Chapter 4 - Sampling techniques to detect and estimate *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) populations infesting dry-cured hams

Abstract

Spatial and temporal dynamics of pest populations is an important aspect of effective pest management. However, absolute sampling of some pest populations such as the ham mite, *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae), a serious pest of dry-cured ham, can be difficult. Sampling techniques were compared and sampling plans were developed for the ham mite. In one experiment, food-baited trap number was maintained at four and mite density varied from 500 to 10,000 individuals in environmentally-controlled 18 m^2 rooms. The number of mites captured in traps increased as mite density increased, indicating a strong positive correlation ($F_{1, 262}$ = 736.32, $P < 0.01$; $r = 0.86$). Experiments conducted in simulated ham aging rooms suggested that mites preferred the proximal to the distal side of the ham. Trap capture also varied with trap location ($F_{3, 124} = 6.88$, $P < 0.01$). There were varying degrees of correlations between trap capture and sampling techniques such as mite counts from the storage rack, vacuum sampling of the floor, and ham sampling to count mites. Correlations between absolute mite counts and trap capture or ham sampling were both moderately strong and significant ($P < 0.01$). Two sampling plans were developed for the mite and it was determined that an economic threshold close to 1 mite/sample unit on the ham can be estimated with 21 or 40 ham samples at precision levels of 0.35 and 0.25 respectively, or with 10 ham samples if classifying the mite's infestation level with respect to an action threshold of 0.43. These findings may give tools for sampling *T. putrescentiae* that can help in the development and evaluation of cost-effective integrated pest management alternatives to methyl bromide.

Introduction

Tyrophagus putrescentiae (Schrank) (Acari: Acaridae) is a storage mite pest of economic importance on dry-cured ham (Graham et al. 2011). It is also a pest of other stored products with moisture content ranging from 15 – 45% (wet basis) and those with high protein and/or fat content such as cheeses, pet food, and cereal-based products. The mite is associated with over 140 commodities (Hagstrum et al. 2013). A typical American dry-cured ham is cured with salt and one or more optional ingredients (USDA 1999), after which the ham is aged in rooms maintained at 24 – 35 °C and 55 – 65% RH for up to two years. These conditions are favorable for the growth and development of the ham mite, *T. putrescentiae* (Graham et al. 2011). There is little or no infestation when the ham is aged for less than three months. The risk of infestation however increases when hams are aged for more than five months. However, short aging duration of less than five months does not assure processors that infestation problems would not occur (Rentfrow et al. 2006). Aging times longer than six months are necessary to obtain the desired product flavor and quality that meets the niche markets (Toldrá et al. 1997).

In the United States, ham mite populations are controlled through the use of the fumigant methyl bromide, which is being phased out in the United States because of its ozone-depleting potential (EPA 2006). More recently, alternative techniques have been explored with potential applications for controlling mites (Phillips et al. 2008, Zhao et al. 2012, Abbar et al. 2013). The development of proper sampling techniques is essential to accurately estimate pest density or infestation level relative to an action threshold (AT) to institute the best management practices possible (Hutchins 1994). The use of baited traps, equipment or product sampling, and vacuum sampling are common indirect methods for sampling and monitoring insect or mite pest populations (Edde et al. 2012, L. Pierce, S. W. Edwards, personal communications) since the

determination of the actual density by direct sampling of pests may not be feasible (Flinn et al. 2009). Although traps can detect infestations earlier and at much lower densities and may require less sampling effort than other sampling methods (Lippert and Hagstrum 1987, Vela-Coiffier et al. 1997, Borges et al. 2011), the type of trap used (e.g. pheromone traps, light traps, etc.), the physiological state of the pest, and other environmental factors can affect pest mobility, and subsequent capture in traps (Hagstrum and Subramanyam 2006). Most storage mite trapping studies have used the BT® trap to detect the presence or absence of mites in different facilities (Thind and Ford 2004, Thind 2005, Wakefield and Dunn 2005, Wakefield 2006). To my knowledge, there had been no detailed trapping and monitoring studies conducted in ham facilities until recently when food-baited traps were used to monitor mite population dynamics in ham aging rooms at commercial ham producing companies in Southeastern United States (Amoah et al. 2016). Visual inspection for the presence of *T. putrescentiae* is common in cured-ham facilities, and facility managers make management decisions based on their observations (S. W. Edwards, personal communication). The use of vacuum to extract mites out of cracks and crevices has been studied for dust mites such as *Dermatophagoides farinae* (Acari: Pyroglyphidae) in homes and the Northern foul mite *Ornithonyssus sylviarum* (Canestrini and Fanzago) (Acari: Mesostigmatidae) in poultry flocks (Kells and Surgeoner 1996, Mitakakis et al. 2002, Tovey et al. 2003), and its adaptation for sampling *T. putrescentiae* has shown promising results (S.A. Kells, personal communication).

Although early detection of an infestation is important, it is equally important to estimate the actual pest density when developing a management program (Savoldelli 2006). Many studies have been carried out with insects and mites to predict actual pest densities with traps and other sampling methods, and also to compare and correlate different sampling methods. (Hossain 1992,

Pereira et al. 1994, Marwan and Allen 2000, Devlin 2001, Palumbo 2003, Musser et al. 2007, Reed et al. 2010, Sánchez and Ortín-Angulo 2011, Buckman and Campbell 2013). Contradictory results regarding correlating trap capture with pest density have been reported (Savoldelli 2006, Buckman and Campbell 2013). Some regression and correlation analyses between trap capture and absolute density of pests in grain samples have explained 25 - 75% of variability for different insect sampling techniques (Lippert and Hagstrum 1987, Vela-Coiffier et al. 1997, Dowdy and McGaughey 1998, Flinn et al. 2009).

Sampling plans have been developed for estimating the densities or determining the infestation levels of stored-grain insects (Hagstrum et al. 1985, Subramanyam et al. 1997, Toews et al. 2003). However, no such studies have been reported for *T. putrescentiae* on dry-cured ham. There is no documented information on how to determine ham mite density, and when to actually perform an intervention based on an action threshold of mite density. Facilities usually follow a calendar-based fumigation plan, or fumigate when they notice two or more mites on surfaces in aging rooms (S. W. Edwards, personal communication). Other facilities, upon noticing mite activity, would spot-clean the area, which is then monitored for approximately 30 days before any further action is taken (L. Pierce, personal communication). The development of a sampling plan for *T. putrescentiae* may therefore help reduce sampling costs, prevent unnecessary pest management actions, and help evaluate the success of management options (Hagstrum and Subramanyam 1996). The objectives of this study were to: (1) determine if varying mite density could affect trap capture of the mite, (2) develop a method to sample mites on dry-cured hams during aging, (3) examine the relationships between relative sampling techniques and actual mite density on dry-cured ham, and (4) develop and validate enumerative and binomial sampling plans for *T. putrescentiae* on dry-cured hams.

Materials and Methods

The standard Trap

The standard trap and bait as described by Amoah et al. (2016) (see Chapters 2 and 3) were used in all experiments. Briefly, the trap is constructed based on the basic design of the BT® trap (Thind 2005), and is made of a plastic Petri dish, 100 mm in diameter and 15 mm high, and spray-painted black on the outside. Eight evenly-spaced holes of approximately 0.5 mm diameter were made about 2 mm above the bottom along the side of the dish to allow entry of mites. The bait is a 10 g circular disc that is approximately 30 mm diameter and 10 mm high that is made of dog food, water, glycerol, brewer's yeast, agar, alphacel, insect vitamin mix, and methyl-p-benzoate solution (see Chapter 2).

Mite culture

Cultures of *T. putrescentiae* were reared at 25 - 30 ºC and 70 - 80% RH on a semi-moist dog food-based diet (Purina Beneful Healthy Puppy, Nestlé Purina Petcare Company, Wilkes-Barre, PA, USA) in 0.95 L glass jars in the Department of Entomology, Kansas State University, USA. These cultures have been in rearing since 2007. All cultures were maintained following the procedures outlined in Chapter 3 and Abbar et al. (2016). The mites that were used were taken from rearing jars that were two to three weeks old from the time the cultures were established.

Relationship between mite density and trap capture

Trapping experiments were conducted in two identical climate-controlled walk-in rooms. These rooms have been used for other studies on *T. putrescentiae* (Amoah et al. 2016, see Chapter 3). The floor of each room was thoroughly cleaned at the end of each experimental replicate, at

least 18 hours prior to the next replicate, by vacuuming and then mopping the entire room with water. The temperature was maintained at 28 ± 2 ^{0}C and controlled with a thermostat and air conditioning/heating unit, while the relative humidity was maintained at approximately 70% using humidifiers (SUL495-UM, Sunbeam Products® Neosho, MO, USA).

A completely randomized design was used for this study. Four traps were used for each replication and the mite density was varied at 500, 1000, and then at 1000-mite increments up to 10,000 mites per replication, making a total of 11 mite densities studied. Each density was replicated six times. The mite density was estimated on the basis that a 0.3 ml volume of mites from a 2-3 week old mite rearing jar contained approximately 1000 nymphs and adult mites. This estimation of mite numbers per volume of mobile mite introduced to a room was used for subsequent densities discussed in this chapter. For each mite density, mites were released in the center of the room, 3 m from both ends and the four traps deployed towards the corners of the room with each trap 2 m from the mite source (Figure 4.1). Traps were collected after 6 h and the number of mites caught in the traps were recorded. Normal distribution of trap capture data were confirmed by subjecting the number of mites captured to PROC UNIVARIATE (SAS Institute 2010). PROC GLM (SAS Institute 2010) was used to determine whether trap captures were affected by mite density. Trap location was also used as a variable, to investigate the possibility that trap capture could differ by their location. The mean captures by location were compared and separated on the basis of the Tukey's Honestly Significant Difference Test to compensate for multiple comparisons only when analysis of variance (ANOVA) was significant at $P < 0.05$. The relationship between trap capture and population density was determined using TableCurve 2D software (Systat Software Inc. 2007).

Mite distribution on ham and comparisons between sampling techniques

Three rooms in Waters Hall at Kansas State University, were set up in July 2015 to mimic commercial ham aging rooms and were maintained at 28 ± 2 °C and 60 ± 5% RH in total darkness. The three rooms varied in size, measuring approximately 3.9 m x 3.5 m, 4.3 m x 2.2 m, and 4.2 m x 2.5 m respectively. A metal rack (Work Choice 5TZ-12, Wal-Mart Stores, Inc.) that was 1.2 m long, 0.5 m wide, and 1.8 m tall was placed in each room. The rack was placed in the center of a marked area of 1.2 x 1.2 m. Each rack had three shelves at heights of approximately 0.6 m, 1.2 m, and 1.8 m from the floor. Three commercial dry-cured hams, (Harper's Country Hams, Inc., Clinton, KY, USA), each weighing 6 - 7 kg were hung from the middle shelf in each room along the mid-line of the shelf and approximately 50 cm apart. A total of approximately 43,200 lab-reared mites were introduced at five locations in each room to create a mite infestation. Approximately 7200 mites were released at each of four of the five locations. These locations were on the floor beneath the rack and on the three shelves. For the fifth location, approximately 14,400 mites were introduced into a container with 300 g semi-moist dog food and placed on the floor. Two experiments were conducted.

In the first experiment, the distribution of mites on the hams were investigated and four sampling techniques were compared. This experiment began 48 h after the initial introduction of mites. Sampling was done on 12 occasions over a 4-week period. The sampling techniques that were tested involved counting mites on the ham surface (ham sampling), on a section of the rack (rack sampling), in floor vacuum samples (vacuum sampling), and inside traps (trapping).

For ham sampling, each ham was divided into two sides referred to as the proximal side, which is the part originally attached to the pig’s body, and the distal side, which is the opposite side (Figure 4.2). Earlier work showed that more mites were distributed about 12 cm above and 6

cm below a hypothetical horizontal line in the middle of the whole ham within six hours of artificial mite infestation of a ham in a pattern of two columns of three rows for six sampling areas (Abbar et al. 2016) (Figure 4.2). One of the two areas in each upper, middle, and lower regions of each side of ham were randomly selected. In each of the three areas, a circular counting area of 23.8 cm^2, which was the area inside a metal lid ring of 5.5 cm diameter from a glass jelly jar (Ball, Wal-Mart Stores, Inc.) was selected as the sample unit and mites within the circle were counted by eye. Rack sampling involved visually counting mites on the outermost metal bar along the length of the lowest and the middle shelves of each rack. Vacuum sampling was done using a battery-powered vacuum cleaner into which a plastic mesh with 0.08 mm opening (9318T22, McMaster-Carr, Elmhurst, IL, USA) was placed (Edde et al. 2012). Two vacuum samples were taken from floor on either sides of the rack along the entire length of the rack and within the 1.2 x 1.2 m marked area at each sampling time. The vacuum cleaner was turned off for approximately five seconds for the sample to settle before opening. The mesh was then transferred into a petri dish and mites collected were counted. Trapping was done by deploying four traps for a period of 6 h, one on the floor directly under the rack, one on the lowest shelf and one on the middle shelf. The forth trap was placed also on the floor 0.5 m from the first trap. Traps were deployed within one hour of taking the ham, rack and vacuum samples.

The mite's preference, if any, for a particular region or side of ham was determined. PROC UNIVARIATE (SAS Institute 2010) was used to determine the normality of the data. The data were then analyzed using PROC GENMOD (SAS Institute 2010), which fits a generalized linear model to the data by maximum likelihood estimation of parameters. The probability distribution was set to a negative binomial and the link function was set to LOG transformation since initial analysis determined that the data were not normally distributed. The means were compared and

separated on the basis of the Tukey's Honestly Significant Difference Test only when the ANOVA was significant at $P < 0.05$. The four sampling techniques were compared whereby sample means for each sampling technique were calculated for each sampling occasion based on the two vacuum samples, two rack samples, four trap samples and 18 ham samples taken in each room. Since there were three rooms and 12 sampling occasions, there were 36 sets of data. Three data sets with missing values and one data set with all zeros were discarded. Therefore, a total of 32 data sets were used. The relationships between the sampling techniques were described using TableCurve 2D software (Systat Software Inc. 2007).

The second experiment began nine weeks after the initial mite introduction to the simulated ham aging rooms. This was four weeks after the first experiment ended. The aging rooms remained unchanged and there was no new mite introduction, but mite population growth was presumed. Five sampling exercises were carried out within two months with a minimum of one week allowed between sampling occasions. This experiment was conducted to correlate trapping and ham sampling, both as described above, with the absolute density of mites. The absolute density of mites was determined through ham washing that involved washing the entire surface of each ham with a jet of water from a wash bottle into a plastic container measuring 40 cm x 27 cm x 13 cm that was placed underneath each ham. Approximately 800 ml of water was used per ham. The liquid that was obtained was transferred into 0.95 L mason jars (85 mm diameter, 160 mm height) for evaluation. Using the Buchner funnel set-up for filtration with a vacuum Erlenmeyer flask, mites were collected on 15 cm diameter black filter papers (Ahlstrom Filtration LLC. Mt. Holly Springs. PA, USA). Suction was maintained until the filter paper appeared dry, after which the funnel was dismantled from the set-up and the filter paper was transferred into a large Petri dish for mite counting. For each sampling occasion, ham sampling was done before ham washing,

and traps were put out within one hour of taking the other samples. Averages for each of the three sampling techniques: trapping, ham surface wash, and ham sampling, were calculated per room for the five sampling occasions. The relationships between the sampling techniques were described using TableCurve 2D software (Systat Software Inc. 2007).

Development and validation of sampling plans

The 32 ham sample data sets obtained from the first experiment as described above were used to develop and validate sampling plans for *T. putrescentiae*. Out of the 32 data sets, 22 data sets were randomly selected to develop Green's fixed precision sampling plan (Green 1970) to estimate mite density, and Wald's Sequential Probability Ratio Test, SPRT, to determine infestation level relative to an action threshold (Wald 1947). The remaining 10 data sets were used to validate the two sequential sampling plans.

Green's fixed precision sequential sampling plan (Green 1970) was used to estimate the density of mites on ham at two precision levels. The mean numbers of mites/sample unit/3 hams/room for each sampling occasion (m) and the variances (s^2) associated with the means were calculated. The means and variances were based on six samples/ham for a total n = 18 samples for each room. In order to model the relationship between the mean and the variance, Green's fixed-precision sampling algorithm requires that the data first be fitted to Taylor's Power Law (Taylor 1961):

$$s^2 = Am^b \quad [1]$$

Both A and b were estimated by regressing sample variances against sample means after log transformation of both variables to $\log_{10}$ scale:

$$\log s^2 = \log a - b \log m \quad [2]$$

where a is the y-intercept, A is the antilogarithm of a and b is the slope. Since a was biased because of the transformation, a correction to calculate A was proposed by Goldberger (1968) as:

$$A = 10^{\log a - 0.5SE(\log a)} \quad [3]$$

where SE(log a) is the standard error of log a from the least-squares regression.

Two fixed-precision stop lines for sequential estimation of density were then calculated as:

$$T_n = [An^{(1-b)}/D^2]^{[1/(2-b)]} \quad [4]$$

where Tn is the cumulative number of mites in the nth sample unit, A and b are parameters of the Taylor's Power Law, and D is the fixed precision level calculated as a ratio of the standard error of the mean to the mean. Precision is defined as the closeness of estimates in repeated measurements or sampling, whereas accuracy is the nearness of a measurement to the actual value of a variable being measured (Zar 1984). Since it is impossible to obtain accurate estimates, precision is measured (Fowler and Witter 1982). A precision level of 0.1 for instance, therefore means that estimates are 90% close in measurements. Precision levels of 0.10 to 0.20 are commonly used in population dynamics studies (Southwood 1978, Hutchison et al. 1988). However, precision levels of 0.25 and 0.35 are used in pest management studies (Subramanyam et al. 1997, Toews et al. 2003). The cumulative numbers of mites in the sample units were plotted against the number of sample using TableCurve 2D (Systat Software Inc. 2007).

The performance of the plan was evaluated using the 10 independent data sets by examining the actual n and D values obtained from sequentially estimating the mite densities at the two fixed precision levels. Resampling for Validation of Sample Plans (RVSP) was used to evaluate the sequential sampling plans (Naranjo and Hutchison 1997). In the program, the minimum sample size was set at 6. Each of the 10 data sets was sampled 500 times (resampling with replacement) to estimate m, s^2, n, and D at the two fixed precision levels.

Wald's SPRT plan allows pest density to be classified relative to an action threshold. The relationship between proportion of samples with one or more mites (y) and mean number of mites/sample unit/3 hams/room (x) based on the 22 randomly selected data sets was described by the following nonlinear regression using TableCurve 2D software (Systat Software Inc. 2007):

$$y = a + b^x \quad [5]$$

The plan is also based on four parameters (Wald 1947): the upper threshold, p_i, the lower threshold, p_o, the probability of exceeding the upper threshold when infestation level is at or below the lower threshold, α, (treating unnecessarily) and the probability of falling below the lower threshold when infestation level is at or above the upper threshold, β (not treating when needed). The dry-cured ham is considered adulterated if ≥1 mite is found (S. Edwards, personal communication, USDA 9 CFR 301). Equation 5 fit to the proportion of infested sample units and mean mite densities indicated that at a density of 1 mite, the proportion of sample units are infested was 0.485. Therefore, in the Wald's SPRT, p_i was set at 0.48. In order to react early, the action threshold (AT) for instituting a treatment was set 5% below *pi* (AT = 0.43). The α and β errors were set at 0.20.

The sampling stop lines were calculated as:

$$Tu = (\text{intercept}) \ln[(1-\beta)/\alpha] + n(slope) \quad [6]$$

$$Tl = (\text{intercept}) \ln[\beta/(1-\alpha)] = n(slope) \quad [7]$$

where *Tu* and *Tl* are the upper and lower sampling stop lines respectively

The intercept was calculated as:

$$1/\{\ln[(p_1 q_0)/(p_0 q_1)]\} \quad [8]$$

The slope was calculated as:

$$\ln(q_0/q_1)/\{\ln[(p_1 q_0)/(p_0 q_1)]\} \quad [9]$$

where:

$q_0 = 1 - p_0$
$q_1 = 1 - p_1$

The intercepts and slopes were obtained through RVSP. Stop lines were generated by plotting *Tu* and *Tl* against *n*.

Results

Relationship between mite density and trap capture

Mite density had a positive relationship with the number of mites caught in traps ($F_{13, 250}$ = 93.13, $P < 0.01$) (Figure 4.3). Significantly fewer mites were captured when mite density was 500 or 1000 when compared to a density of 10,000. At densities of 2000 to 4000, significantly fewer individuals were captured than at densities of 5000 to 8000. There was a positive nonlinear relationship between mite density and the trap capture ($F_{1, 262}$ = 736.32, $P < 0.01$; $r = 0.86$) (Figure 4.3). Trap capture was consistent among trap locations for each density with the exception of mite density of 10,000 where the highest trap capture was found in trap location 4 with the least in location 2. Trap capture at location 4 (318.3 ± 18.9 mites/trap) was not different from traps at location 1 (266.7 ± 18.9 mites/trap) or 3 (273.7 ± 23.5 mites/trap) but was significantly different from trap location 2 (195.8 ± 26.6 mites/trap). Trap locations 1, 2, and 3 were not significantly different from each other.

Mite distribution on hams and comparisons between sampling techniques

The distribution of mites on the surfaces of hams varied among rooms ($F_{2, 586}$ = 41.71, $P <$ 0.01) with room 2 recording the highest number of mites per sampling unit and room 3 recording the least number. The two sides of the ham, proximal and distal, were also significantly different. More mites were recorded on the proximal side of the ham compared to the distal side ($F_{1, 586}$ = 52.13, $P < 0.01$). There were no significant differences among the top, middle, and lower ham regions for each side of the ham ($F_{2, 586}$ = 0.20, $P = 0.82$). Trap capture was significantly different for each location relative to the rack ($F_{3, 124}$ = 6.88, $P < 0.01$). Traps on the floor underneath the

racks captured the highest number of 39.6 ± 13.04 mites per trap and was significantly different from the other three locations which were not different from each other.

A range of correlations between sampling techniques were determined. Weak correlations were recorded between ham surface sampling and rack sampling ($r = 0.26$; $P = 0.14$) (Figure 4.4), between ham sampling and vacuum sampling ($r = 0.2$; $P = 0.30$) (Figure 4.5), and between trapping and rack sampling ($r = 0.35$; $P = 0.05$) (Figure 4.6). Medium correlations were determined between trapping and ham surface sampling ($r = 0.4$; $P < 0.05$) (Figure 4.7) and between trapping and vacuum sampling (0.57; $P < 0.01$) (Figure 4.8). The highest correlation recorded was between vacuum sampling and rack sampling ($r = 0.74$; $P < 0.01$) (Figure 4.9). The relationships between the absolute sampling from total ham washing and two relative sampling techniques, ham sampling and trapping, were also determined. There were moderate correlations between absolute density and ham surface sampling ($r = 0.47$; $P < 0.01$) (Figure 4.10) and also between absolute density and trapping ($r = 0.61$; $P < 0.01$) (Figure 4.11).

Development and validation of sampling plans

Green's Sequential Sampling Plan

The log variance against log mean regression for ham sampling showed that the variance increased significantly with mean density with $a = 0.401 \pm 0.04$, $b = 1.65 \pm 0.09$ and $r^2 = 0.95$. After applying the Goldberger (1968) correction, $A = 2.497$. The slope, b of 1.65 ± 0.09 reflects the aggregation nature of mites on ham (Figure 4.12). Green's sequential sampling plan precision lines are presented in Figure 4.13 and validation of the plan presented in Table 4.1. More samples would be needed for the estimation of mean density at the precision level of 0.25 in comparison to the precision level of 0.35. Stop lines for both precision levels converge as population densities

become lower and the number of samples needed approached similar levels (Figure 4.13). The number of sample units required to estimate a density of 1.056 mites/sample unit (close to economic threshold of 1 mite/sample unit) can be estimated with 40 samples at *D* of 0.25 and with 21 samples at *D* of 0.35 (Table 4.1). The RVSP simulation also showed that the overall mean precision levels obtained were less than the intended precision levels 70% of the time, making the observed precision levels worse than expected for only three out of the 10 densities: 0.278, 2.222, and 9.389 mites/sample unit. This occurred because the predicted sample variances for the three densities were up to three times less than the observed variances. At the higher precision level of 0.25, the number of samples required was higher, ranging from 19 to 65, whereas the number of samples required at precision level of 0.35 ranged from 10 to 34. Overall, Green's fixed precision sampling plan indicated that a mite density of 1 can be estimated, and this sampling plan can be used for estimating mite densities at the two precision levels.

Wald's Sequential Probability Ratio Test

The relationship between mite density and proportion of infested ham samples was established through nonlinear regression. The proportion of infested samples increased with increase in mite density. The proportion of samples infested at density of 1 mite/sample unit was 48.5% (Figure 4.14). The lower and upper stop lines for classifying the infestation level of the mite relative to an action threshold are shown in Figure 4.15. The lower stop line intersects the x-axis at 10 sample units. The Operating Characteristic (OC) function was near unity when $pi \leq 0.3$ (Figure 4.16). The OC functions were 0.7801 and 0.19 at the lower threshold and upper threshold respectively. At $pi > 0.48$, the OC function decreased from 0.19 to zero. At the AT, the OC function was 0.46. Although the nominal errors were set at 0.2, the actual errors generated by RVSP were determined from the OC curve (Figure 4.16). The 1 - OC value at *po* gives the actual α

of 0.219; the OC value at *pi* gives the actual β of 0.1902. The Average Sample Number (ASN) curve (Figure 4.17) indicated that to classify *pi* between 0.085 and 0.823, an average of 11 to 39 sample units need to be examined.

Discussion

Results indicated that trap capture increased with increase in mite density and suggest that mite numbers in traps could be used to estimate mite density in a room. Buckman and Campbell (2013) studied the effect of density on the capture of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) in pheromone traps and reported that the number of beetles captured in traps increased significantly as beetle density increased although the proportion of beetles captured remained consistent. However, contradictory results were obtained by Savoldelli (2006) who reported a decrease in trap efficiency with increasing number of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) males. Although it was not expected that there would be variation in trap capture among trap locations for any mite density, trap captures for mite density of 10000 varied significantly, indicating that subtle differences in the trapping environment used here may have influenced trap captures (Buckman and Campbell 2013).

In the simulated ham aging rooms it was determined that the distribution of *T. putrescentiae* on the ham was higher on the proximal side which is rougher in texture, compared to the distal side, which is smoother and oilier with lard. The rougher proximal side may be providing the mites with better anchorage and protected harborage. In addition to surface roughness, the presence of smooth and uniform lard on the distal side may be a contributing factor in determining the mite's preference for the proximal side. Lard has been reported to prevent population growth of *T. putrescentiae* under laboratory conditions (Abbar et al. 2016), and in Spain, coating hams with hot lard is a common practice to control mites in dry-cured ham (García 2004). These conditions may therefore make the proximal low-lard side more suitable in supporting mite survival and reproduction.

Trap counts in the simulated ham aging rooms suggest that mites are more active on the floor compared to higher up on the shelves. A previous study (Chapter 3) found that the mite may be positively geotactic. Geotaxis has been reported to influence the distribution of some stored grain insects such as *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) (Jian et al. 2002, Jian et al. 2006).

Sampling ham mites is difficult due to the small size of the mite. Visual examination of hams for mites may be labor intensive and ineffective as mites tend to hide and reproduce in cracks and crevices on hams. Therefore population build-up may go unnoticed. Vacuum sampling from floors collected the highest number of mites, which may support an earlier conclusion that mites are positively geotactic. However, evaluation of vacuum samples may be more technical, difficult, and time consuming than other sampling techniques because a lot of debris may be collected with the mites, and separation of mites from debris may be cumbersome. Trapping could be the sampling technique of choice since it is inexpensive, quick, easy to use, and does not require the presence of an operator except to deploy and collect the traps. Trap capture of a pest varies depending on factors such as trapping duration, number of traps deployed, trap location, presence of refugia, pest aggregation behavior, physiological state, density, and environmental conditions as reported in several studies on storage pests (Mullen and Dowdy 2001, Savoldelli 2006, Hagstrum and Subramanyam 2006, Fedina and Lewis 2007, Buckman and Campbell 2013, Amoah et al. 2016). Although there was a strong positive correlation between trapping and actual mite density in a clean room when there was no source of food in the initial experiment for this study, the relationship between trapping and actual density in the aging rooms was not as strong. However, trapping provided better population estimates than ham surface sampling. Approximately 37% of the variation in trap capture can be explained by the relationship between trap capture and absolute

mite density on ham. This again supports earlier findings about the possible positive geotactic behavior of the mites. Many factors have been shown to significantly affect trap capture rates of storage pests so it is therefore not surprising that other studies achieved similar relationships between trap capture and actual density of insect pests. For example, Lippert and Hagstrum (1987) recommended regression of the ratio of average trap catch divided by the average number of insects per 0.2 kg grain sample against the number of insects per trap, and this model could explain 37% of the variation in their dataset. Additionally, Vela-Coiffier et al. (1997) used linear regression between the number of insects captured in a probe trap and absolute density determined with a single 0.3 kg deep bin cup sample or 0.7 kg tier sample, and could account for 25-34% of variability for *C. ferrugineus*.

Results suggest that Green's sequential sampling is useful for estimating the density of *T. putrescentiae* at or near the economic threshold, or in cases for which the RVSP variance from resampling is at or below the predicted variance from Taylor's Power Law. The stop lines for estimating the density at the fixed precision levels indicated that the number of sample units required to cross the stop lines decreased as pest density increased. The least amount of sampling is therefore required, as one would expect, when population levels are at high or low densities, with more intense sampling needed at intermediate population levels closer to the economic threshold. Results from Wald's SPRT showed that the lower stop line intersects the x-axis at 10 sample units. Therefore, a minimum of 10 sample units should be examined for classifying the mite's infestation level with respect to the AT of 0.43. The OC curve indicated that the binomial sampling plan performed well in classifying the infestation levels of the mite with respect to the AT. The actual α and β of 0.219 and 0.1902 respectively suggested that with an α that is greater than the pre-set value of 0.2, one would take unnecessary action more than 20% of the time when

in fact an action is not required. A lower β suggested that one will fail to take action less than 20% when an action is required. The errors were essentially equal to the preset errors.

Southwood (1978) suggested that for Green's sampling plan, a precision level of 0.25 is adequate for management activities. However, the availability of resources and other factors may determine the practical level of sampling that can be reasonably accomplished. However, due to the physical size of an individual mite, counting can be cumbersome. It may be easier to use Wald's sampling plan to classify infestation level relative to an AT since it was only the presence or absence of mites that was determined, and this requires fewer samples to make a decision. Although the data here compared well with those from other studies (Subramanyam et al. 1997, Toews et al. 2003), it would be desirable to further test the applicability of the sampling plans presented in this controlled laboratory study to sampling in commercial ham facilities. The work reported here is the first to develop and suggest useful sampling plans for *T. putrescentiae* on dry-cured ham, and these sampling plans can now be applied to field validation in commercial facilities.

In summary, this study identified the distribution pattern of *T. putrescentiae* on dry-cured ham, compared and correlated sampling techniques, and developed sampling plans for the mite. While most of the sampling methods examined correlated weakly or moderately with each other and with absolute mite density, all have practical advantages and disadvantages. The use of sampling techniques for the detection of storage mites may still be in its infancy so the relationship between these sampling techniques and actual population levels need more research especially for *T. putrescentiae* on dry-cured ham and alternative control options need to be developed. Also, the use of sequential sampling plans for the ham mite may help avoid inappropriate pest mitigation

activities, as well as avoid a lack of action for presumed undetected pest populations that are truly present and increasing in size.

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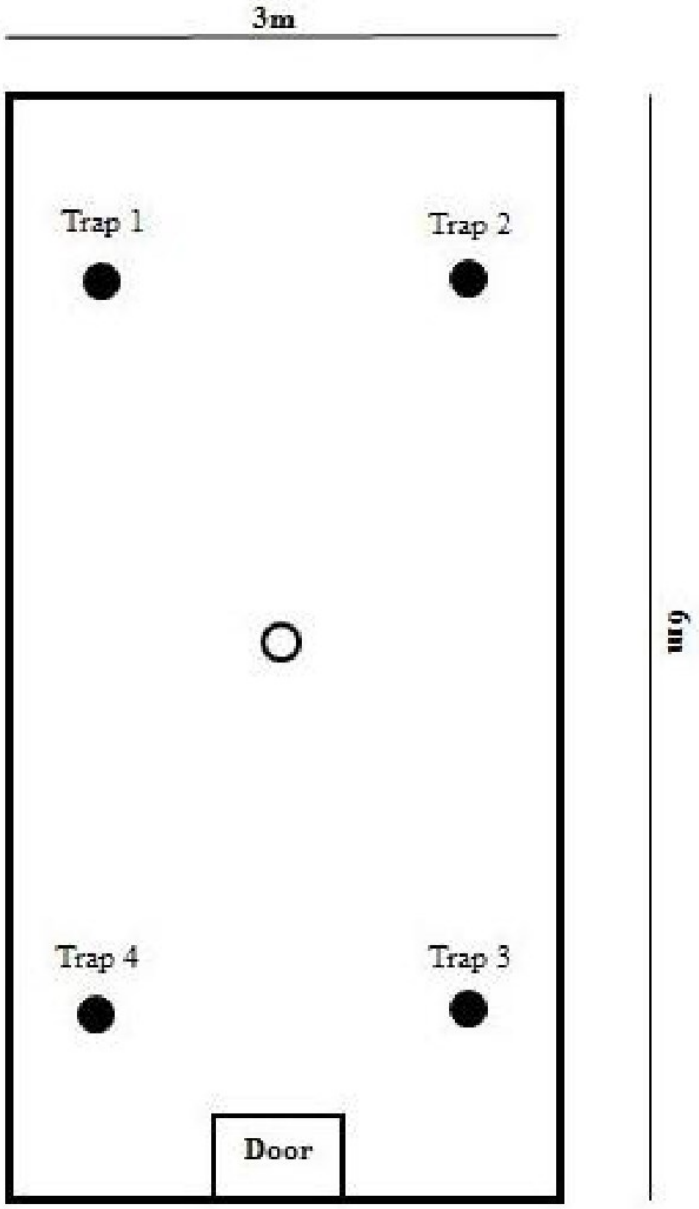

Figure 4.1 Experimental chamber details (Not drawn to scale).

The four black circles represent the four traps and their respective locations. The central circle is the mite release point for each replication.

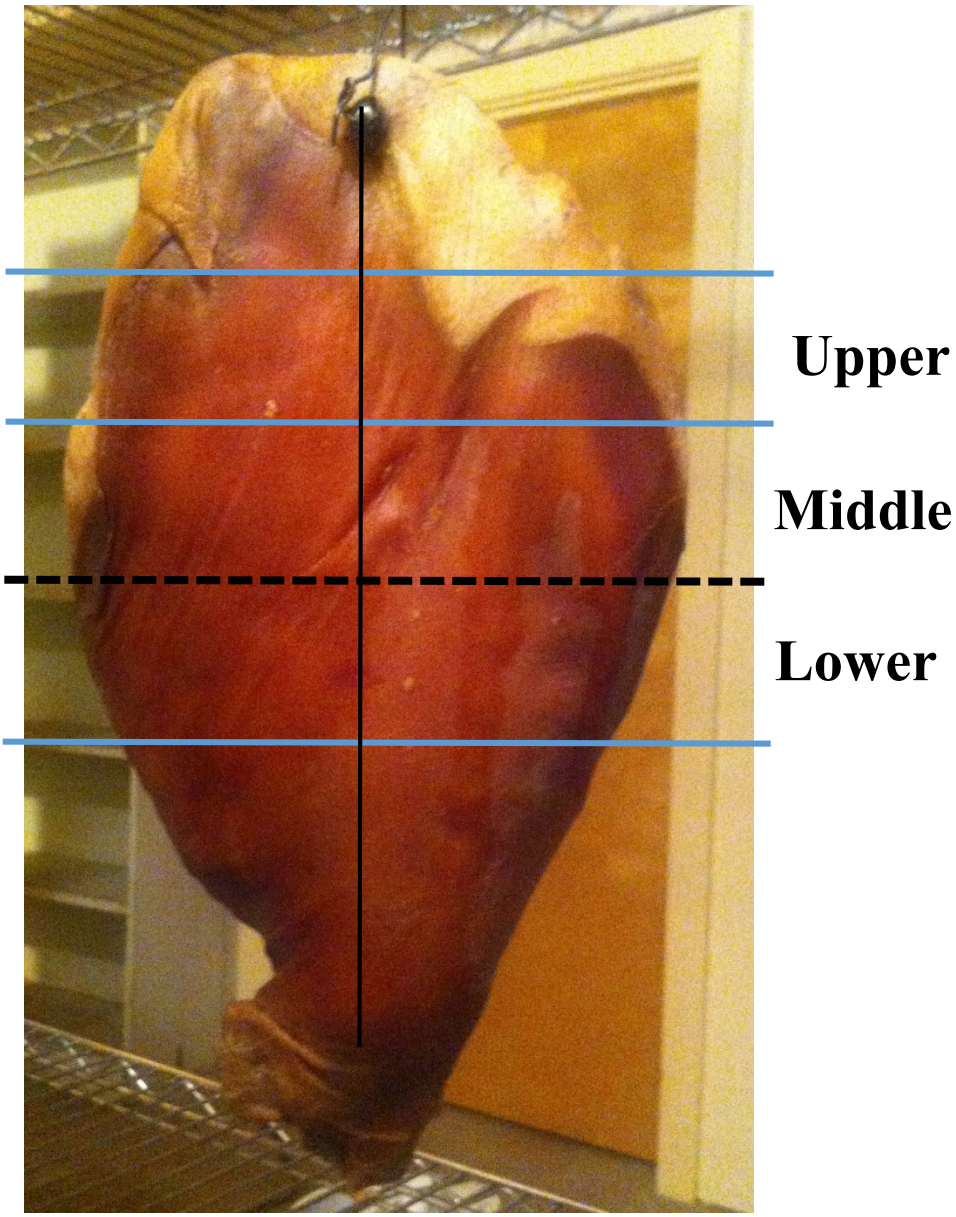

Figure 4.2 Distal side of dry-cured ham showing regions from which mites were counted.

– – – . Hypothetical horizontal line dividing ham into two.

——— Hypothetical vertical line further dividing ham into six regions.

A similar demarcation was done for the opposite (proximal) side of the ham.

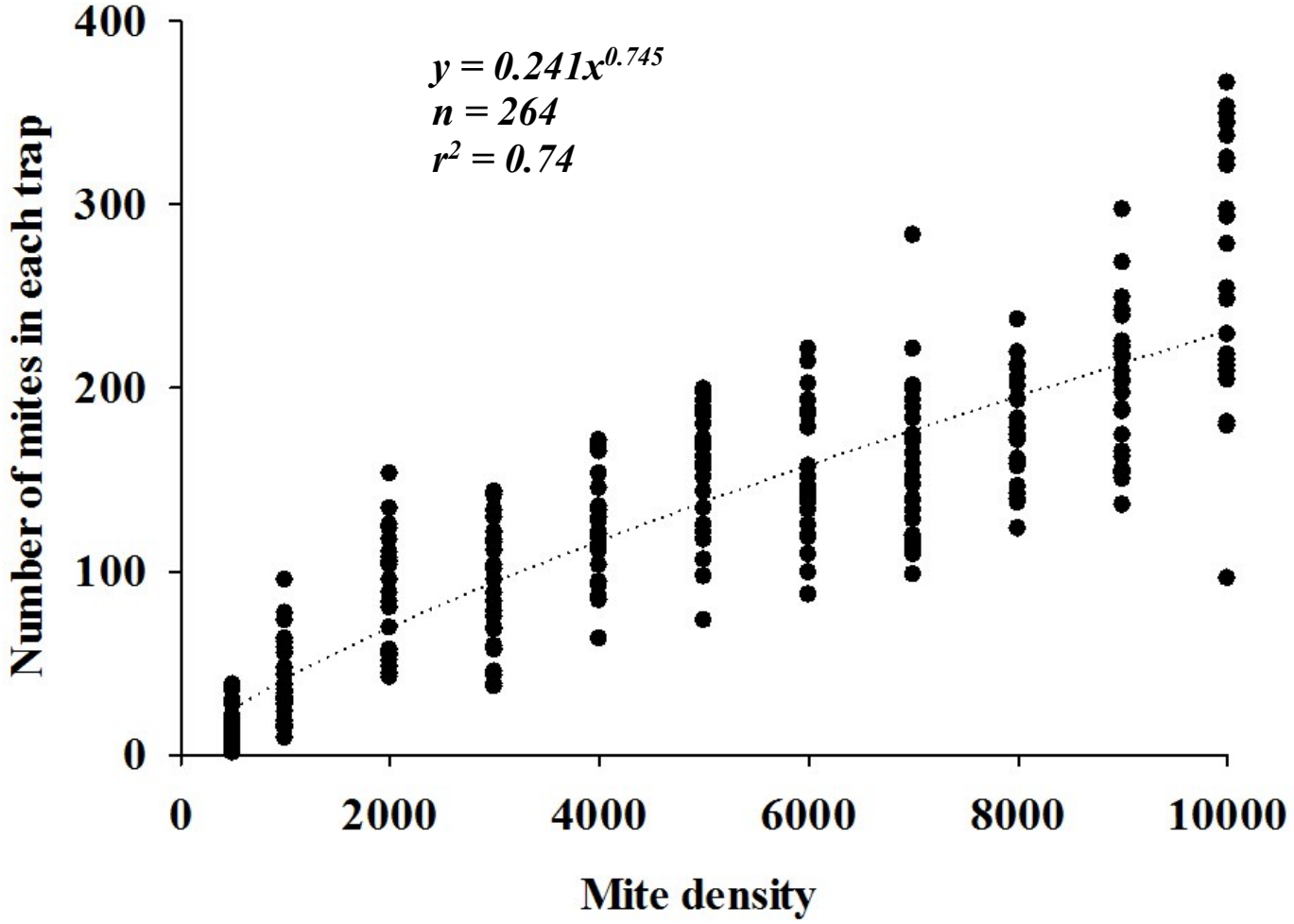

Figure 4.3 Correlation between *T. putrescentiae* density and trap capture under controlled conditions.

Dotted line is the regression line and the circles represent the number of mites in each trap for each mite density ($P < 0.01$).

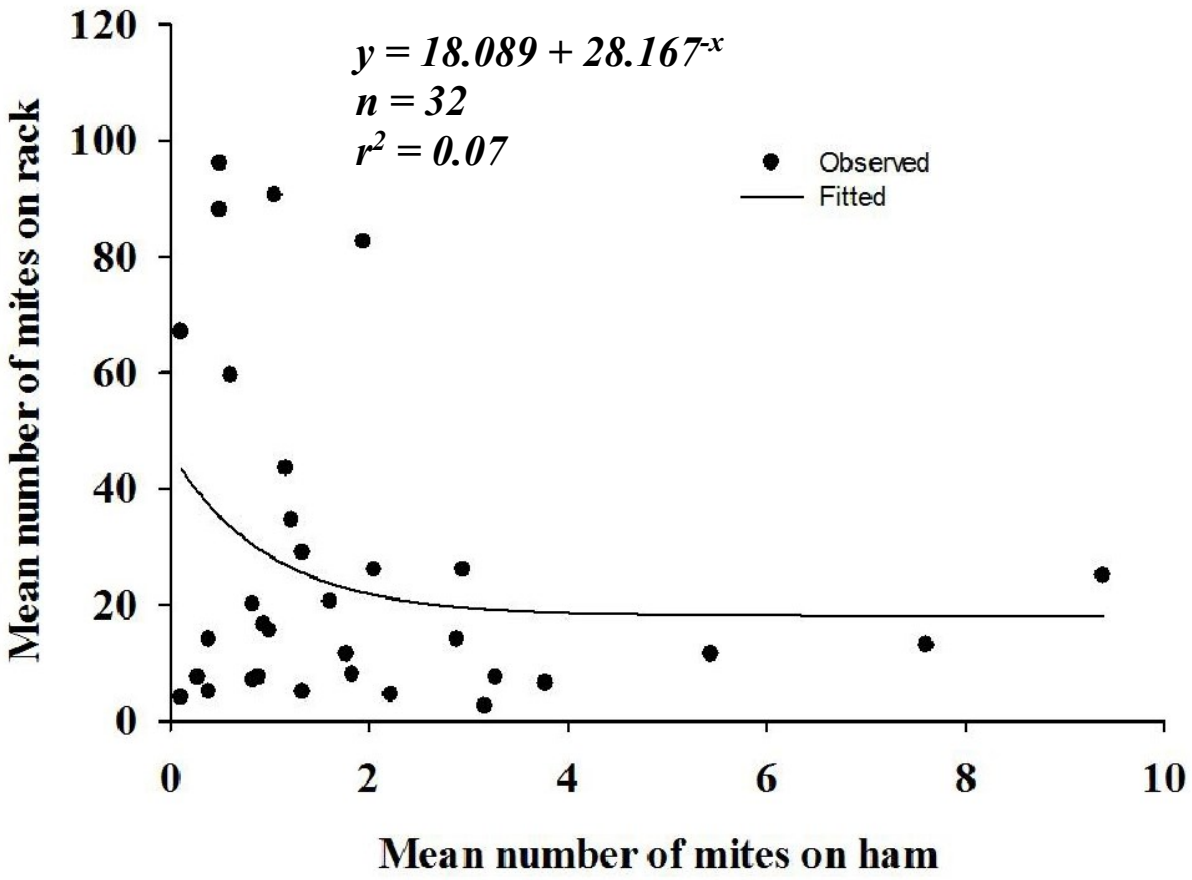

Figure 4.4 Correlation between rack sampling and ham sampling of *T. putrescentiae.*

Observed values were averages for each of the three simulated ham aging rooms for each sampling occasion.

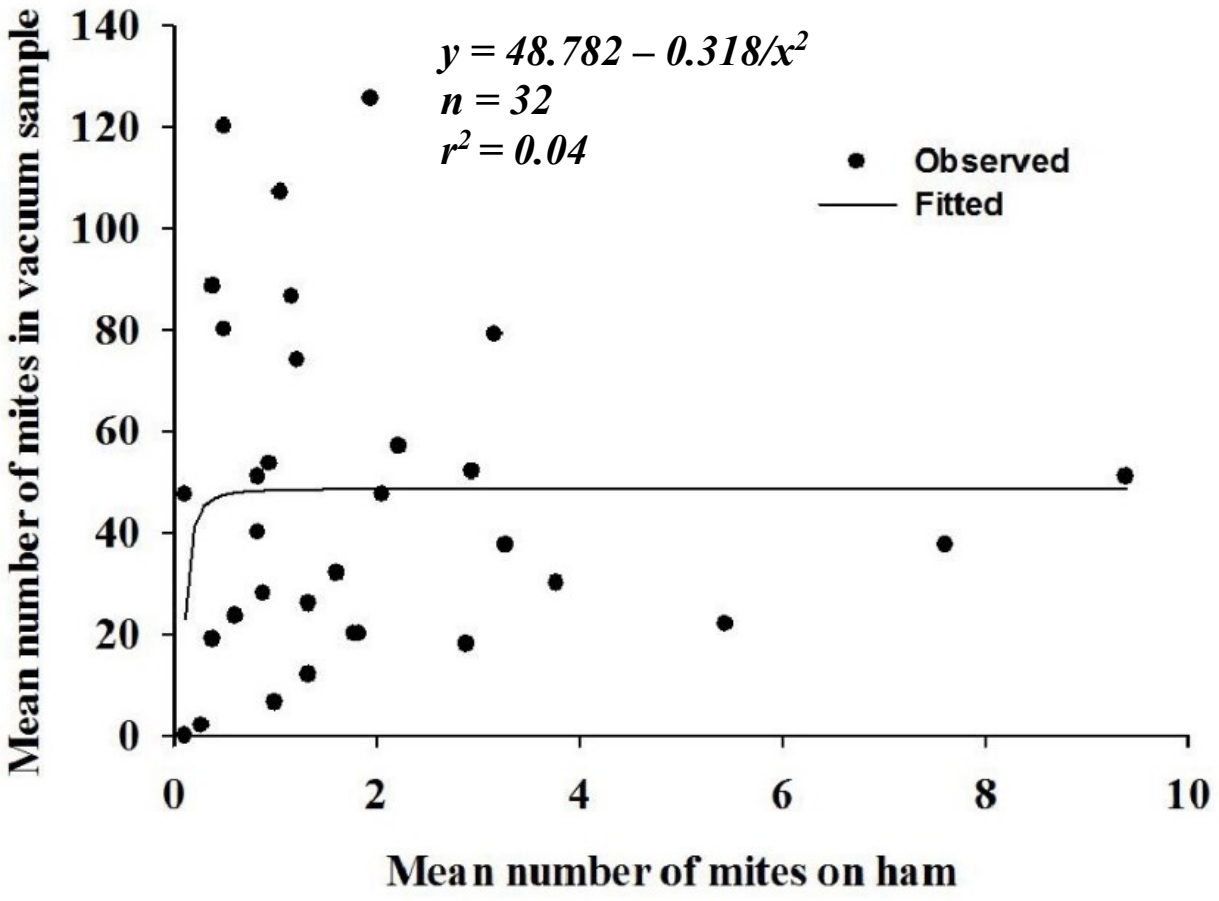

Figure 4.5 Correlation between vacuum sampling and ham sampling of *T. putrescentiae.*

Observed values were averages for each of the three simulated ham aging rooms for each sampling occasion.

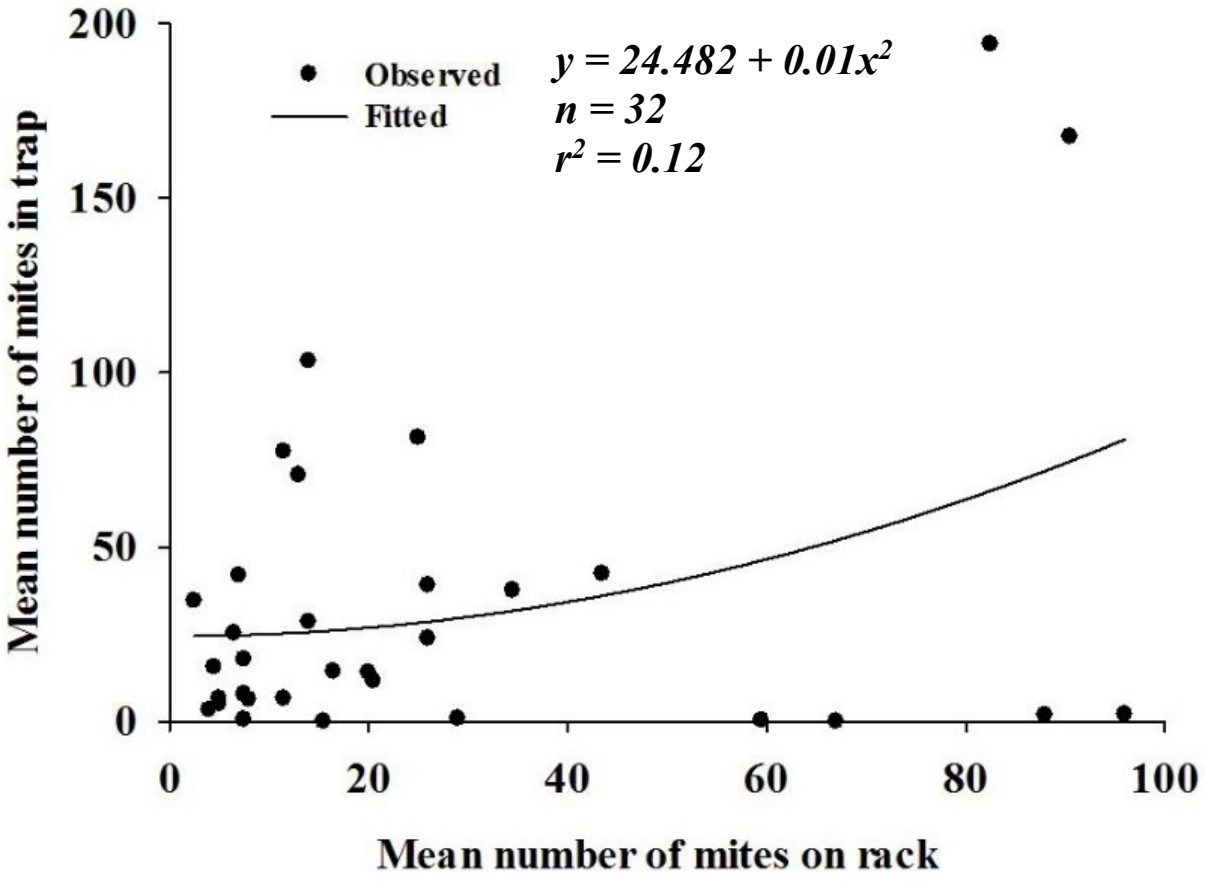

Figure 4.6 Correlation between trapping and rack sampling of *T. putrescentiae.*

Observed values were averages for each of the three simulated ham aging rooms for each sampling occasion.

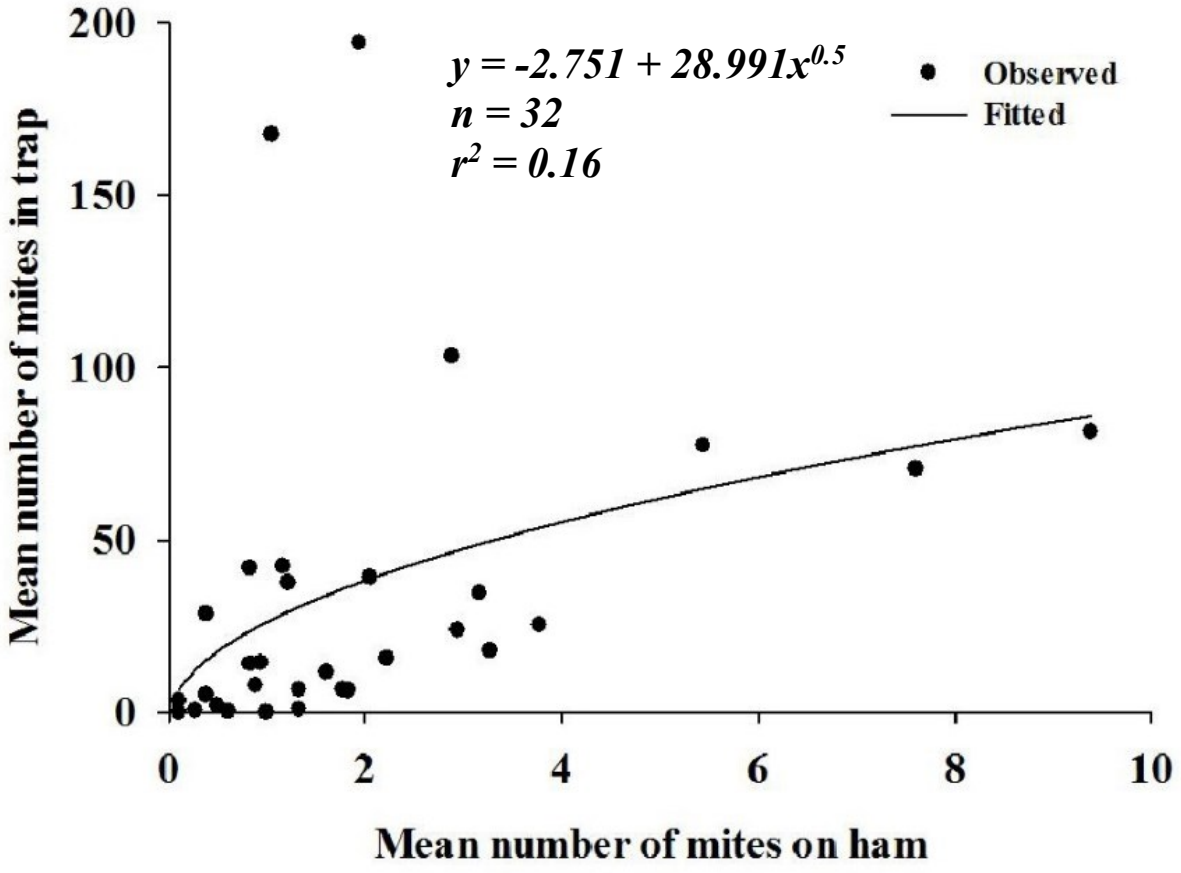

Figure 4.7 Correlation between trapping and ham sampling of *T. putrescentiae.*

Observed values were averages for each of the three simulated ham aging rooms for each sampling occasion.

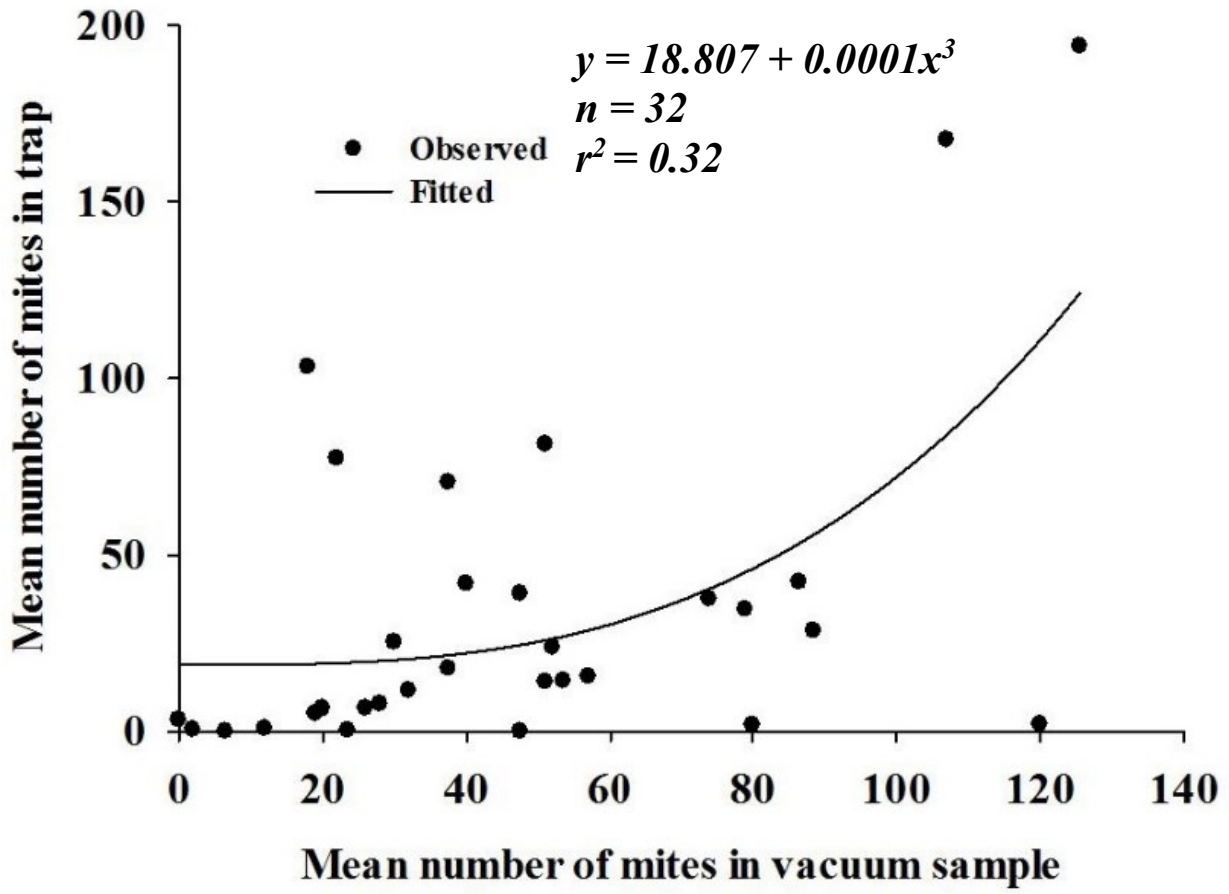

Figure 4.8 Correlation between trapping and vacuum sampling of *T. putrescentiae.*

Observed values were averages for each of the three simulated ham aging rooms for each sampling occasion.

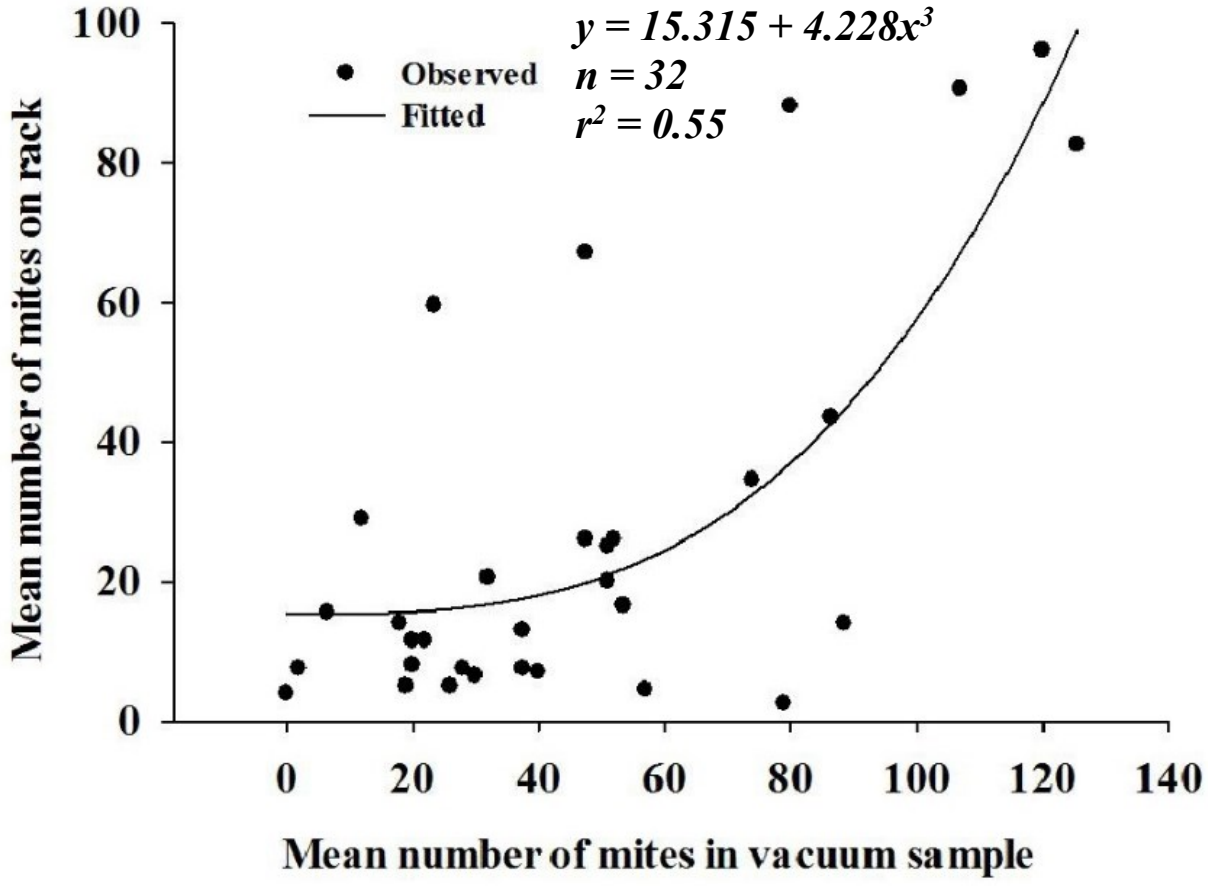

Figure 4.9 Correlation between rack sampling and vacuum sampling of *T. putrescentiae.*

Observed values were averages for each of the three simulated ham aging rooms for each sampling occasion.

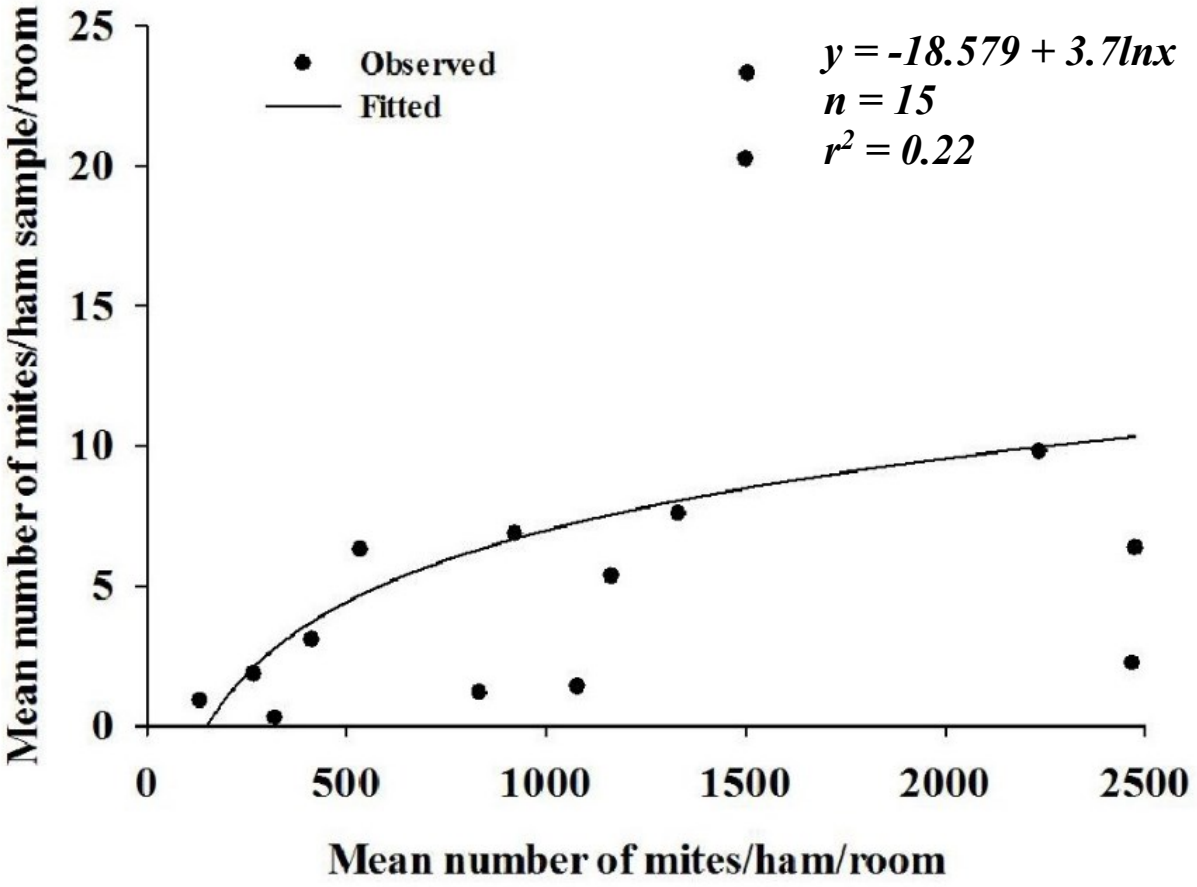

Figure 4.10 Correlation between ham sampling and absolute mite density on ham.

Observed values were averages for each of the three simulated ham aging rooms for each sampling occasion.

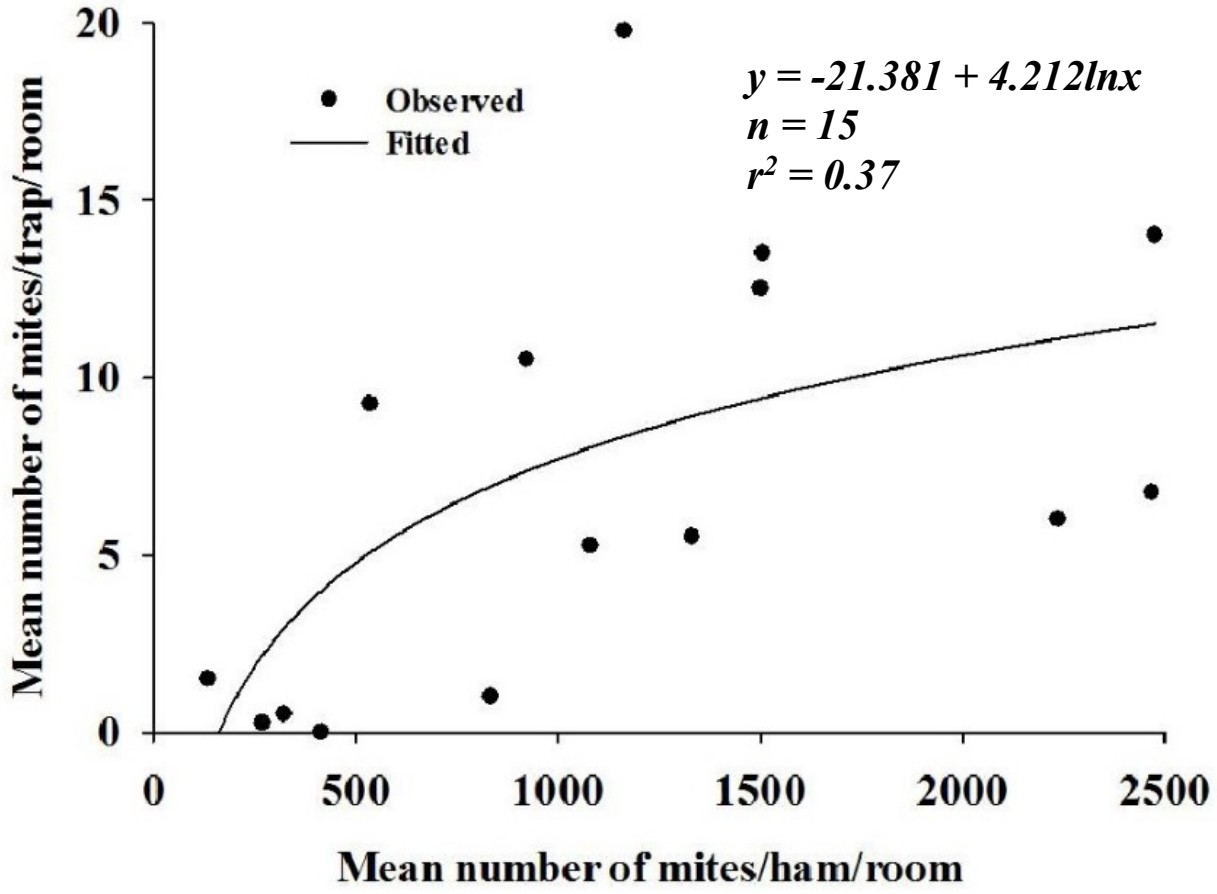

Figure 4.11 Correlation between trapping and absolute mite density on ham.

Observed values were averages for each of the three simulated ham aging rooms for each sampling occasion.

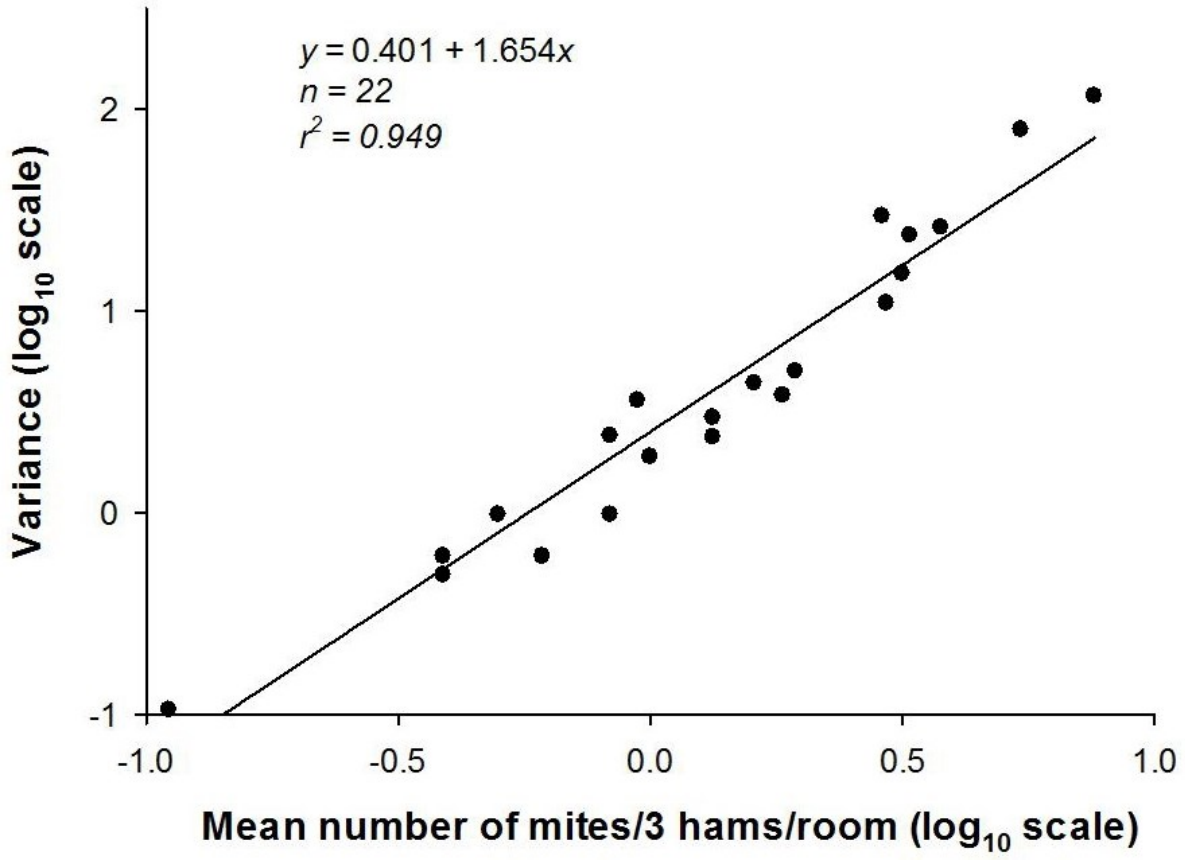

Figure 4.12 Linear regression showing relationship between log variance and log mean for *T. putrescentiae.*

Observed values were averages for each of the three simulated ham aging rooms for each sampling occasion ($P < 0.01$).

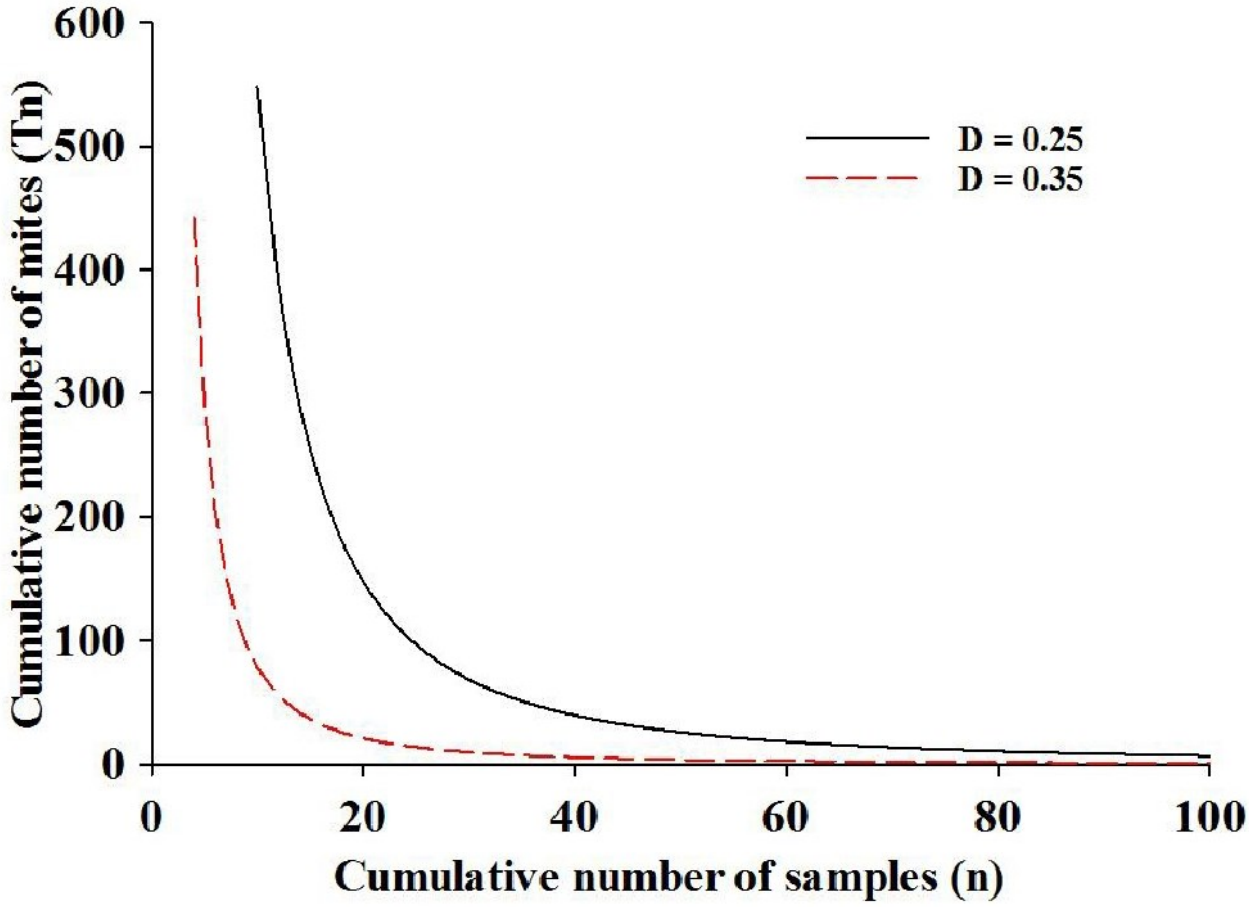

Figure 4.13 Green's Fixed Precision stop lines for sequential estimation of number of *T. putrescentiae* per ham.

The lines were drawn using 22 data sets randomly selected from 32 data sets that were averages for each of the three simulated ham aging rooms for each sampling occasion.

Table 4.1 Performance of the fixed precision sequential sampling plan for estimating the density of *T. putrescentiae.*

				Fixed Precision			
				***D* = 0.25**		***D* = 0.35**	
Data set	***m*[a]**	***S*^{2}[b]**	**Predicted *S*^{2}[c]**	**n[d]**	**Observed *D*[e]**	**n[d]**	**Observed *D*[e]**
1	0.278	0.918	0.301	65	0.43	34	0.59
2	0.500	0.735	0.790	52	0.23	27	0.32
3	0.889	1.751	2.055	42	0.22	22	0.30
4	1.056	1.820	2.732	40	0.20	21	0.27
5	1.167	1.441	3.224	39	0.16	20	0.22
6	1.222	1.595	3.479	38	0.16	20	0.23
7	1.778	5.124	6.469	33	0.21	18	0.30
8	2.056	4.500	8.225	32	0.19	17	0.25
9	2.222	14.183	9.353	31	0.29	16	0.39
10	9.389	162.016	101.422	19	0.30	10	0.41

[a] Sample mean expressed as mean number of *T. putrescentiae*/sample unit (5.5 cm dia. area). Each mean is based on 18 sample units.

[b] Sample variance.

[c] The predicted sample variance was calculated as Am^b, where $A = 2.497$, $b = 1.654$ and m is the sample mean.

[d] Mean number of sample units required to estimate m as determined by the RVSP software.

[e] Observed mean precision level for estimating m as determined by the RVSP software.

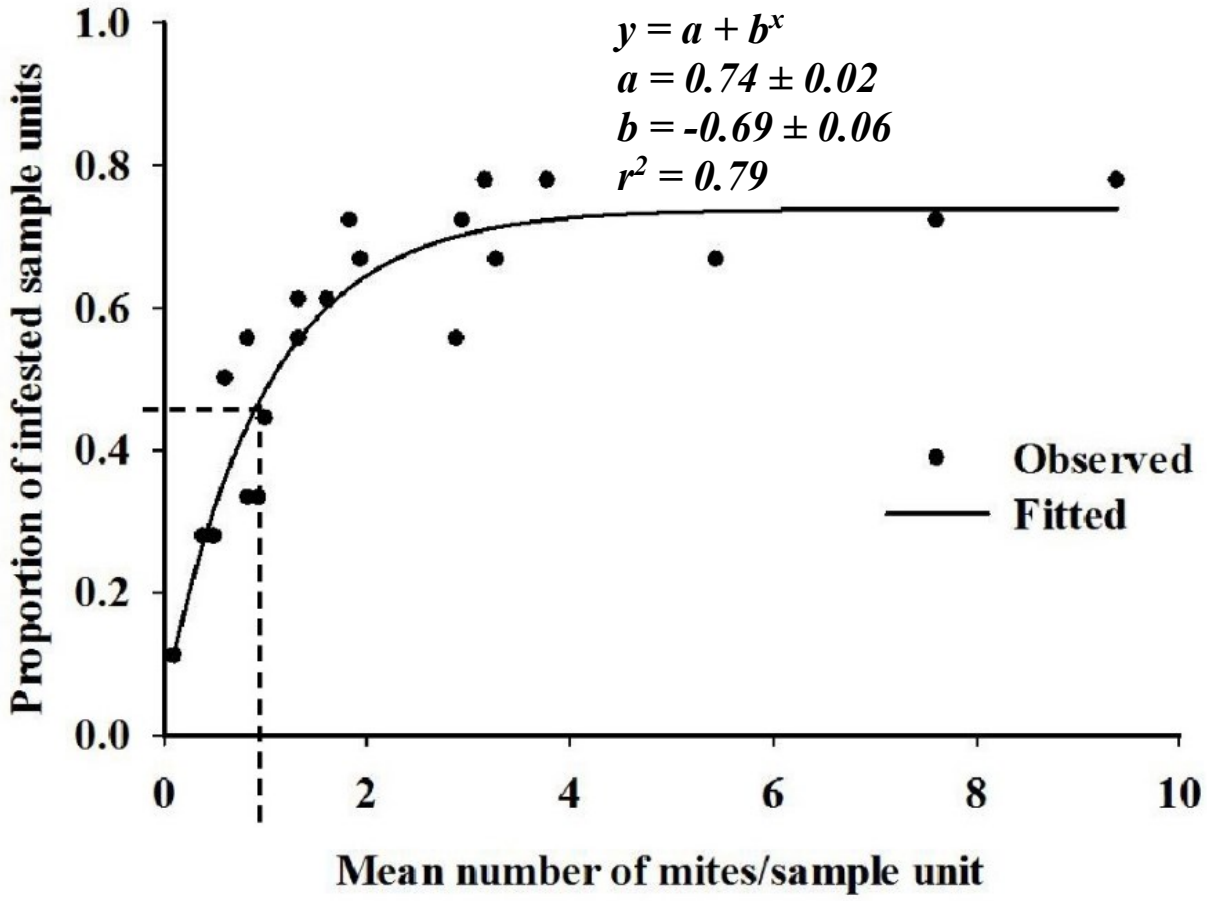

Figure 4.14 Non-linear relationship between *T. putrescentiae* density and proportion of sample units infested with one or more *T. putrescentiae*.

Observed values were the 22 data sets randomly selected from 32 data sets that were averages for each of the three simulated ham aging rooms for each sampling occasion. Dashed lines indicate the proportion of sample unites infested with a mite.

Table 4.2 Observed and resampled statistics for Wald's Sequential Probability Ratio Test Sampling Plan for *T. putrescentiae.*

	Observed Statistics		Average statistics over 500 simulations	
Data set	**PI**[a]	**Mean**[b]	**PI**[c]	**n**[d]
1	0.111	0.278	0.085	11
2	0.333	0.500	0.294	33
3	0.500	0.889	0.550	39
4	0.556	1.056	0.614	28
5	0.611	1.167	0.670	19
6	0.722	1.222	0.772	12
7	0.611	1.778	0.673	19
8	0.722	2.056	0.780	12
9	0.556	2.222	0.605	28
10	0.778	9.389	0.823	10

[a] Actual proportion of sample units infested with one or more mites

[b] Sample mean expressed as mean number of *T. putrescentiae*/sample unit (5.5 cm dia. area). Each mean is based on 18 sample units.

[c] Proportion of sample units infested with one or more mites based on 500 simulations with resampling.

[d] Number of sample units required to estimate the mean based on 500 simulations with resampling.

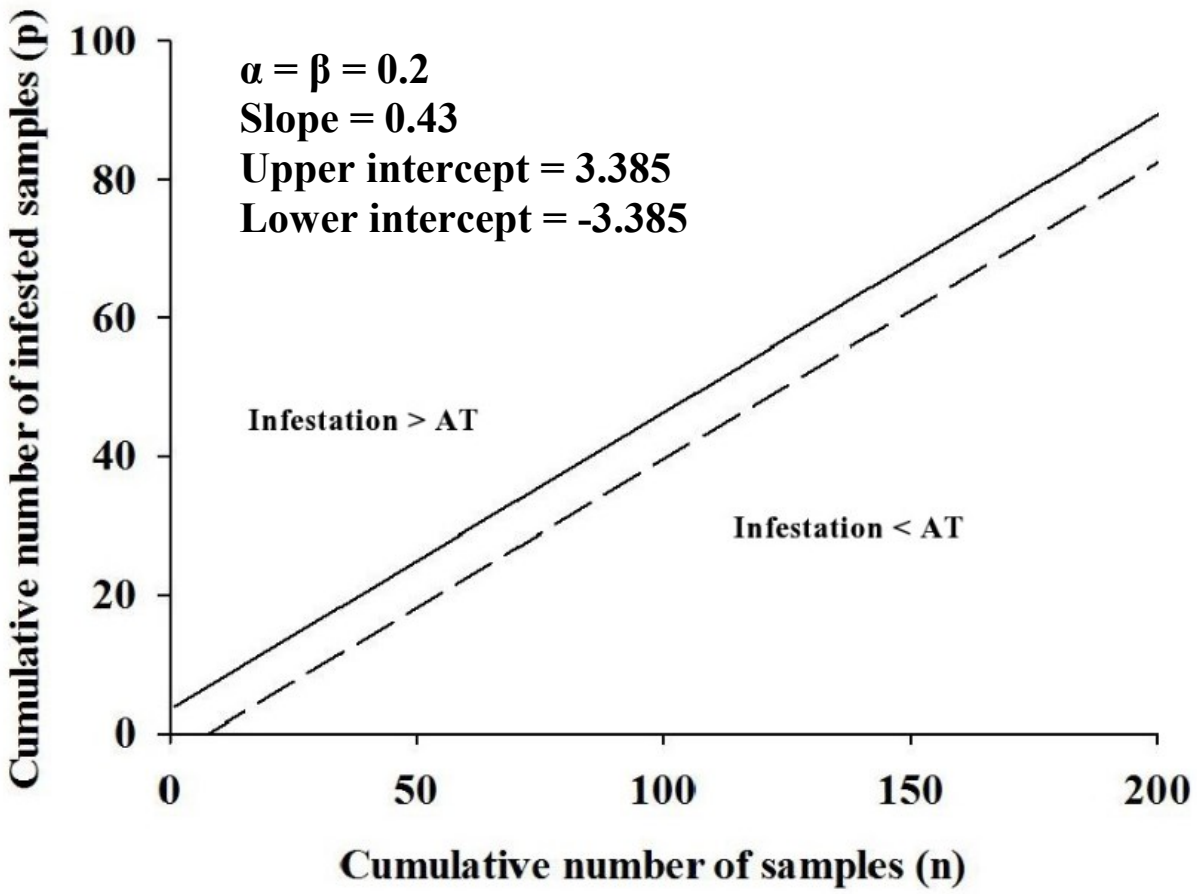

Figure 4.15 Wald's Sequential Probability Ratio Test stop lines for classifying the infestation level of *T. putrescentiae* relative to an action threshold (AT).

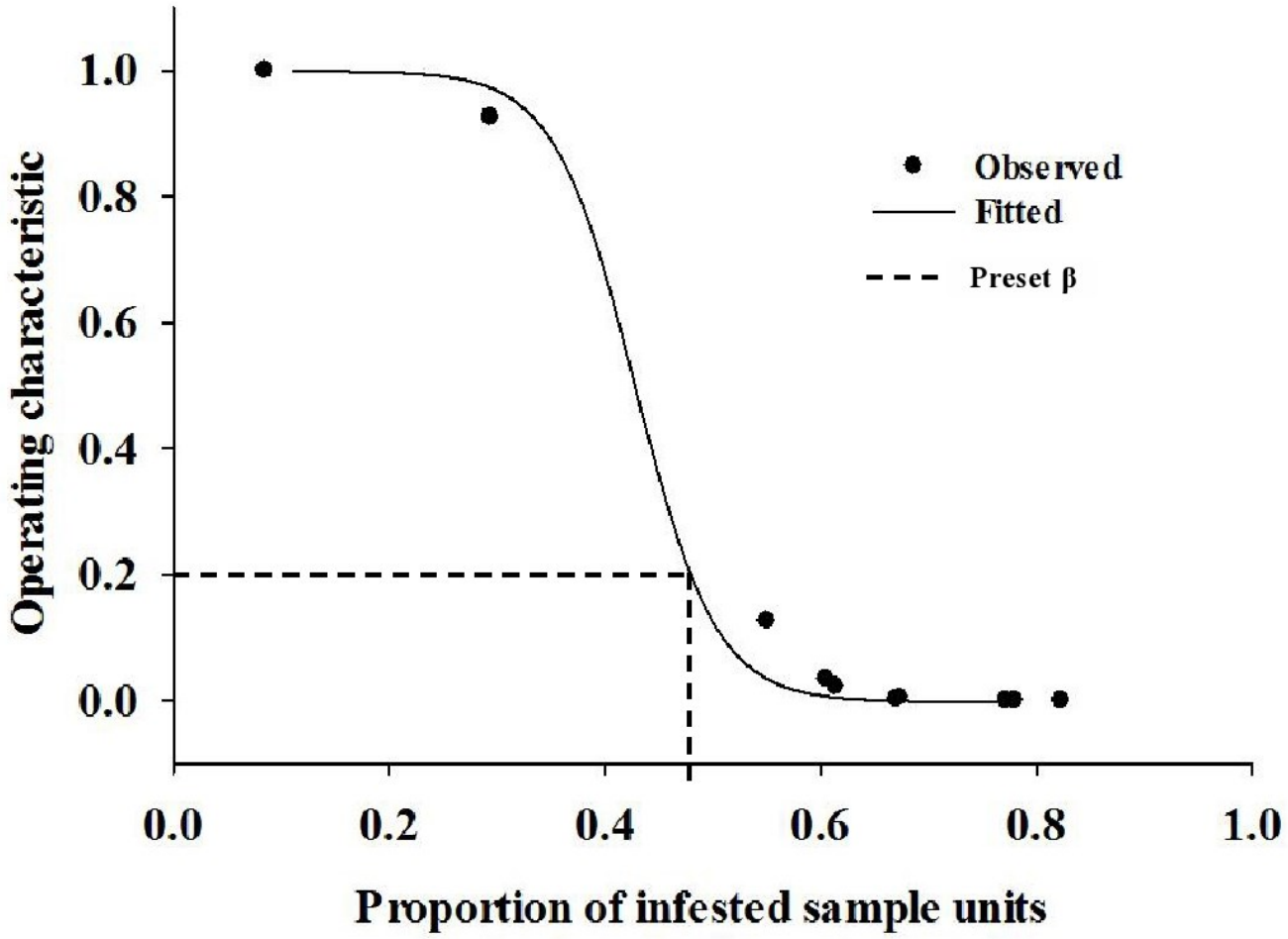

Figure 4.16 Operating characteristic curve for the binomial sequential probability ratio test sampling plan.

($\alpha = \beta = 0.2$, $p_o = 0.38$, $p_1 = 0.48$).

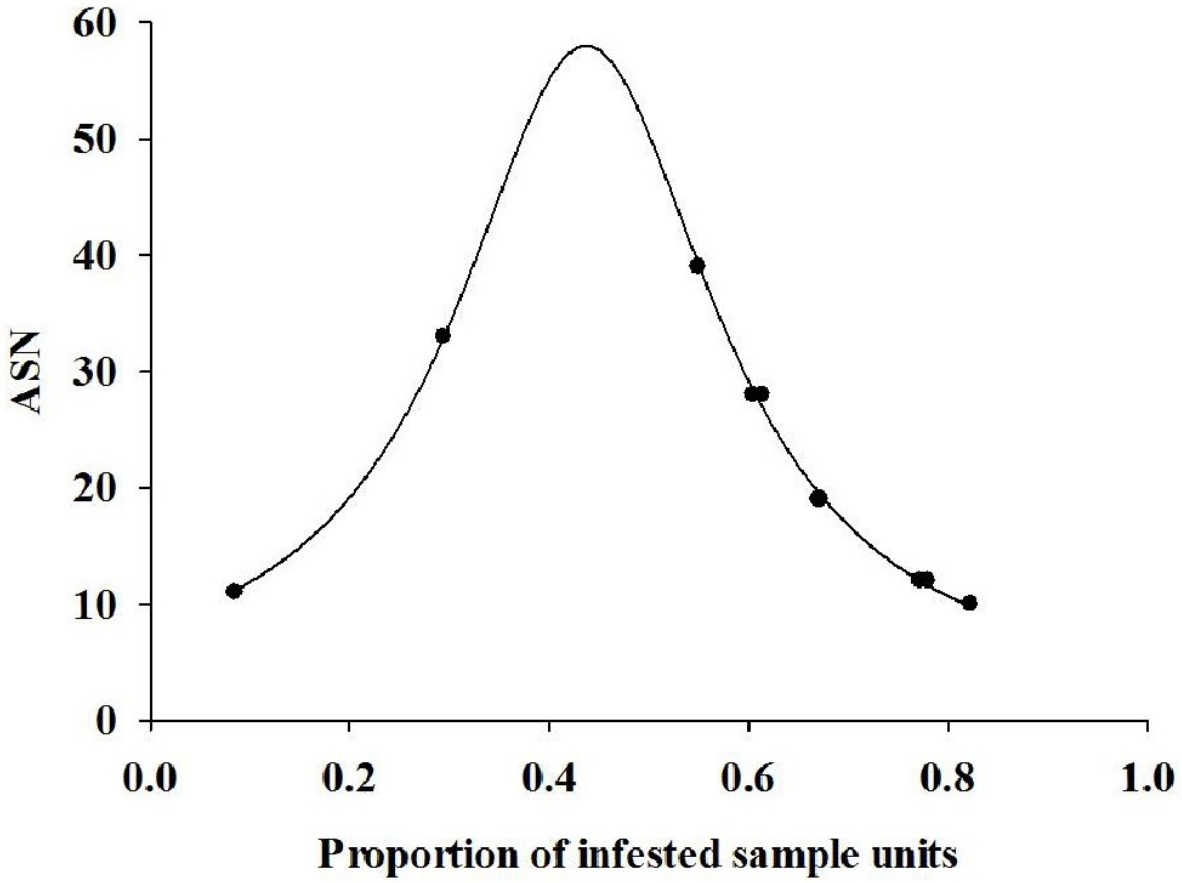

Figure 4.17 Average sample number (ASN) curve for the binomial sequential probability ratio test sampling plan.

($\alpha = \beta = 0.2$, $p_o = 0.38$, $p_1 = 0.48$)