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MUSHROOM FLIES AND FARM WORKERS: FLY ECOLOGY AND VECTOR COMPETENCY AND IPM IMPLEMENTATION AMONG HIPSANIC MUSHROOM FARMWORKERS.

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ABSTRACT

The mushroom sciarid fly, *Lycoriella ingenua* and the mushroom phorid fly *Megaselia halterata* are important pests in commercial mushroom farming in the U.S. The Mushroom Sciarid fly has been anecdotally associated with the occurrence of the fungal pathogen *Trichoderma aggressivum* ft. *aggressivum* in commercial mushroom farms. It has been reported that female *L. ingenua* are attracted to this fungus in mushroom compost for oviposition. The research presented in this dissertation shows that *L .ingenua* benefits from its attraction to the fungus and provides experimental evidence for the mushroom sciarid flys' acquisition and mechanical vectoring of *T.aggressivum* spores, proving that the association between organisms is mutual.

The ecology and behavior of the mushroom phorid fly outside mushroom farms in the U.S. has not been studied. This dissertation presents data on the flight activity, distribution and behavioral aspects of *M. halterata* in and around commercial mushroom farms in Chester County, PA. Specifically, flight activity and mating behavior in relation to the time of day. Results have implications for fly management such as timing of farm activity to improve fly exclusion and the use of mating disruption techniques. *M. halterata* population dynamics within commercial mushroom crops is characterized and factors related to fly density is analyzed and discussed.

Mushroom Integrated Pest Management is crucial to the success of mushroom farming in PA. The success of IPM depends on the extent to which it is implemented by farm owners and farmworkers alike. A study where the effect of mushroom farmworkers perceptions around mushroom pests and diseases on farmworker IPM behaviors is presented. Results show that high risk and control perceptions around mushroom pests and pathogens rather than knowledge on IPM predicts whether farmworkers will engage in IPM behaviors on the job. These results have applications for the development of IPM intervention programs among mushroom farmworkers.

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Chapter 1

Introduction

World production of cultivated, edible mushrooms has increased more than 30-fold since 1978 (from about 1 billion kg in 1978 to 34 billion kg in 2013) (Royse, 2013). Although China is currently the number one producer of the white button mushroom, (*Agaricus bisporus*) the United States holds second place. Volume of sales from 2017-2018 totaled 917 million pounds and the value of sales was $1.23 billion. The state of Pennsylvania is the number one producer of *A. bisporus* in the US accounting for 65% of the national production and generating 1.2 billion dollars in sales in 2016-2017 (USDA, 2018).

The first person to successfully grow the white button mushroom *A. bisporus* in Chester County, Pennsylvania, was William Swayne in 1885. Swayne was a florist who was looking to do something with the space beneath his greenhouse benches. After the success of his idea, he set out to build the first mushroom farm in the area. Mushrooms sold at an attractive price in city markets and the operation was working, making others quickly follow into the mushroom growing business ("Mushroom Farmworkers of Pennsylvania", n.d.).

Flies as pests in modern mushroom production

Almost half a century after the beginning of commercial mushroom farming in Pennsylvania came the first reports of mushroom insect pests in the scientific literature. Mushroom insect pests include species from three dipteran families: Sciaridae, Phoridae, and Cecidomyiidae, the latter being considered a secondary pest. Among the Sciaridae, the predominate fly species in North America is *Lycoriella ingenua* (Dufour) (syn. *L. mali, L. solani*)

while in the UK both *L. castanescens* (Lengersdorf) (syn. *L. auripila*) and *L. ingenua* are mushroom pests (Menzel and Mohrig, 2000; Fletcher and Gaze, 2008). Among the Phoridae, *Megaselia halterata* (Wood) is predominant in the U.S, while *Megaselia nigra* (Meigen), *Megaselia bovista* (Gimmerthal) and *M., halterata* (Wood), have been reported in Europe, the latter also being the most prominent on commercial mushroom farms. The research presented in this thesis focuses on the North American mushroom sciarid fly, *L. ingenua* and the mushroom phorid fly, *M. halterata*. The earliest report of the mushroom phorid fly invading commercial mushroom farms in Europe was in 1953, while in the U.S the first report was in 1975, although Broekhuizen reported the species to be associated with fungi in 1938 (Broekhuizen,1938).

Sciarids

Most of sciarid and phorid mushroom fly research has been conducted in the context of commercial mushroom farming. Studies have focused on fly life tables, host preferences and to a lesser extent on fly ecology within *A. bisporus* mushroom growing conditions for pest management purposes. Life tables and host preferences have been studied in relation to the mushroom growing substrate or mushroom compost. For example, the sciarid mushroom flies can successfully complete development on the mushroom compost alone (compost lacking *A. bisporus* mycelia), whereas mushroom phorid flies, being obligate fungal feeders, cannot. Further, which stages of *A. bisporus* colonization of the compost and what *A.bisporus* strains are preferable to both each fly species have been characterized, which is useful for calculating the timing of fly infestations and fly control measures (Kielbasa & Snetsinger 1981; Cantelo & San Antonio 1982, Smith et al., 2006).

The biochemistry and microbiology of the mushroom growing substrate is a field of study in its own right. Drawing from that research, Cloonan explored the chemical ecology of the mushroom scarid fly by studying microorganisms affecting the attraction and reproductive success of *L. ingenua*. He found a specific relationship between the sciarid mushroom fly and the

microbiome of the mushroom growing substrate. One microorganism that was a focus of his research was the causal agent of mushroom green mold Disease, the pathogenic fungus *Trichoderma aggressivum* ft *aggressivum* (Cloonan et al., 2016; Cloonan et al., 2017). There is a body of anecdotal evidence that the occurrence of this disease is related to sciarid fly population densities on mushroom farms (personal communication). Cloonan found that gravid female *L. ingenua* flies are attracted to compost infested with this fungus as opposed to healthy, un-infested compost and the mushroom itself (Cloonan et al., 2016). This finding concurs with other research in insect chemical ecology that has confirmed that host microorganisms are often the drivers behind insect attraction, rather than the host itself. This research also provided evidence in support of the anecdotally reported relationship between the Mushroom Scarid fly and green mold disease in mushroom farming. Two questions about this association remained unanswered: Are there fitness-related benefits driving *L. ingenua* attraction to green mold and does the sciarid fly actually vector green mold disease in commercial mushroom farms (as suggested by mushroom growers),. These questions are addressed in chapters two and three of this dissertation.

Phorids

Recently, mushroom phorid fly (*M. halterata*) populations have markedly increased from minor to major pest status on mushroom farms in Chester County, Pennsylvania (personal communication). Growers conjecture that phorids are responding to the loss of previously available insecticides. These phorids tend to leave the farms ‘en mass’ and invade nearby residential communities causing a nuisance for residents and a public relations problem for the mushroom industry (personal communication). Mushroom growers have declining access to effective chemical products against mushroom phorid flies mainly because of new pesticide regulation resulting from the Food Quality Protection Act and the inconsequential size of the mushroom industry in pesticide industry product development and marketing. In chapters 4 and 5 I present research on the ecology and population dynamics of *M. halterata* on commercial

mushroom farms in Chester County, Pennsylvania. Currently, only three papers on *M. halterata* in mushroom farms exist (Hussey, 1965; Binns et al., 1979; Navarro et al., 2001) . These studies are based on data gathered on mushroom farms in Europe. They reveal aspects of *M. halterata* annual phenology, flight activity outside mushroom farms according to temperature and daylight and population dynamics within mushroom crop cycles. While valuable, weather patterns and mushroom farming techniques in Europe differ from those in the U.S. Given the recent mushroom phorid fly outbreak in Pennsylvania, in Chapter 4 I address behavioural aspects of *M. halterata* outside commercial mushroom farms (immediate farm surroundings) in Chester County, PA that may render useful in designing future fly control techniques for the area.

Research on *M. halterata* inside commercial mushroom farms in the U.S. was conducted by Rinker (1982), as part of a pest management research program implemented by Penn State in Chester County, PA. Rinker's work focused on the biology and damage potential (economic injury level) of *M. halterata* as well as the fly's relationship with an endoparasitic nematode (Rinker, 1982). My dissertation is the sole published work North America for over thirty years that includes aspects of mushroom phorid fly ecology in commercial mushroom farms. In chapter 5 I present research conducted inside commercial mushroom farms which focuses on *M. halterata* population dynamics within the mushroom crop cycles using updated fly sampling techniques than those utilized by Rinker. This includes variables that may influence the levels of *M. halterata* population density on commercial mushroom farms. This dissertation aims to elucidate factors that can affect fly control.

IPM implementation for Hispanic component of the mushroom industry

Finally, Mushroom Integrated Pest Management in PA has long relied on preventative measures aimed at avoiding the establishment and spread of pests and diseases. The success of

these measures relies on the consistency with which they are applied by farm owners and farm workers alike. In this dissertation I present research using the Health Belief Model (HBM) on what influences whether Hispanic mushroom farmworkers in PA engage in mushroom pest and disease prevention behaviors. The HBM has been widely applied to pesticide safety behaviors among Hispanic farm workers in the U.S (Vaughn 1993; Arcury et al., 2002; McCauley et al., 2002; Snipes 2009). Its theoretical premise is that risk mitigating behaviour is affected by a persons' subjective appraisal of a risk, which is modified by external cues such as education, information training etc. In addition to this, for a risk mitigating behaviour to occur, a high sense of internal control or self-efficacy must exist. That is, the belief that one can personally take action to mitigate a risk (Arcury et al., 2002). Farmworker perceptions on the risks of pesticide exposure and on their locus of control over avoiding exposure are taken into account in order to develop educational materials and training methods aimed at pesticide safety. Pesticide safety is seen by public health professionals as a matter of environmental justice, since farmworkers are exposed to pesticides, which they have no control over. I believe that IPM can be viewed as a matter of environmental justice as well. When implemented efficiently, IPM can ultimately reduce exposure to pesticides, through reduced applications of these chemicals. Farmworkers must be taken into account and listened to in order to develop appropriate IPM interventions. These interventions must address farmworker perceptions on pests and pathogens as well as on the feasibility of prevention measures. Here I apply the HBM for the first time to explore farmworker perceptions on the risk of mushroom pests and pathogens. I use the model to predict and explain 'IPM behaviors' among mushroom farmworkers. I do so while encouraging education and extension professionals as well as farm owners to address the issues keeping farmworkers from implementing IPM.

Overview of chapters

Chapter one of this thesis reviews the most important relevant scientific literature on aspects of *L. ingenua* and *M. halterata* behavior and ecology in both laboratory and commercial mushroom production settings. The chapter also reviews risk perception research, particularly, that which utilizes the Health Belief Model (HBM) is also reviewed. Reviewed literature includes the theoretical framework of the HBM as well as how it has been applied to pesticide risk and control perception research among Latino farmworkers in the U.S.

Chapter two is a manuscript published in The Journal of Pest Science, titled "The mushroom fly *Lycoriella ingenua* benefits from its association with green mold disease (*Trichoderma aggressivum* f. *aggressivum*) in commercial mushroom production". In this chapter I determined whether there are fitness differences between flies reared on *T. aggressivum*-infected spawned mushroom compost versus spawned compost without *T. aggressivum*. Through rearing experiments, I found that there are fitness related benefits when *Lycoriella ingenua* fly develops on spawned compost infested with green mold disease as opposed to non-infested mushroom compost and fully spawned mushroom compost. Benefits include higher adult emergence rate, faster development time from larva to adult and larger adult females. I discussed the possible mechanisms behind the green mold-related benefits, such as improved nutrition and substrate pre-digestion provided by the green mold fungus or the suppression of *A.bisporus* defenses against *L.ingenua* when parasitized with *T. aggressivum*. I concluded that my findings emphasize the management of both organisms that are possibly associated to each another.

Chapter three contains a series of experiments I conducted to determine whether the female sciarid fly is a vector of *T. aggressivum* spores. I reported that gravid *L.ingenua* flies mechanically vector green mold disease by landing on the fungal colonies, physically acquiring the fungal spores and spreading them to new substrates through further movement. In addition, I

showed that female flies vector larger amounts of green mold after a pre-oviposition period of 36 hours as opposed to a shorter period of 18 hours. I also showed that the sciarid larvae vector green mold through the consumption and excretion of the spores, which remain viable in the larval frass. I concluded that there is a mutualistic association between *L. ingenua* and *T. aggressivum*. While we know that *L.ingenua* is attracted to mushroom green mold and benefits from the consumption of the fungus, through my findings we now know that the green mold benefits from the association through its ability to colonize new areas through the movement of the fly.

In chapter four I explored the distribution and flight activity of the mushroom phorid fly, *M. halterata* outside commercial mushroom farms through trapping experiments with using yellow sticky traps. I found that *M. halterata* focused its flight activity over turf areas rather than windbreaks or spent compost piles outside of mushroom farms, possibly for mating purposes. I also reported that flight activity is highest in the afternoon until midnight at higher temperatures yet at lower temperatures activity ceases after sunset. I concluded that spent mushroom compost is not a source of fly re-infestation since the adults are not attracted to this substrate provided it has been steam pasteurized prior to removal from the growing house. I also concluded that fly exclusion can be improved by focusing farm operations around temperature ranges and daylight times when fly activity is at its lowest.

Chapter five consists of a one year study done on 14 commercial mushroom farms in Chester County, PA. I describe general fly population dynamics within these farms and conduct a regression analysis to elucidate which farming practices and location factors are related to the differing fly population densities on the farms. I concluded that *M. halterata* life cycle from egg to adult within commercial mushroom farming conditions is completed in 23-25 days. Overlapping generations as well as two generations within one farming cycle can occur, depending on the length of the cropping cycle. Farming factors such as the type of compost used

and organic certification were not related to *M. halterata* population densities on farms. The type of construction of the farm and the density of neighboring mushroom farms, as well as double steaming practices, were related to *M. halterata* population densities.

Chapter six is a mushroom pest and disease risk and control perception study among Latino mushroom farmworkers. With data from 105 surveys, farmworkers risk and control perceptions were found to be generally high. A logistic stepwise regression revealed that risk and control perceptions are positively correlated to the frequency in which farmworkers engage in mushroom integrated pest management behaviors, whereas basic IPM knowledge, is not. I conclude that extension efforts to promote IPM on mushroom farms must highlight the level of susceptibility of mushrooms crops and the farm economy towards pests and diseases. Efforts must also be aimed at providing farmworker's with a better sense of control over pest and disease prevention, both at the individual and team level.

Literature review

Commercial mushroom farming

The state of Pennsylvania is the number one mushroom producer in the U.S.(Pecchia & Beyer, 2013) Mostly, the white button mushroom (*Agaricus bisporus)* is cultivated. Pennsylvania mushroom farming is performed in a closed environment, inside farms consisting of multiple growing rooms termed *doubles.* In conventional farms, which are constructed with cement blocks, growing rooms are separated from each other by way of a wall and often do not share the same roof. Each room has a two doors located at opposite end of the room. One opens to a breezeway and is used for human traffic (i.e. the entrance of harvesters or crop managers). The second door (located at the opposite end of the room), opens to a wharf (composting area), this door is used for

heavy operations such as filling the rooms with compost or casing and emptying the room. Inside each growing room are wooden structures containing the beds (shelves) on which the mushrooms are grown. The substrate used for *Agaricus* mushroom growing is termed mushroom compost or mushroom growing substrate.

The mushroom farming process and mushroom integrated pest management efforts

The mushroom farming process begins with the preparation of the mushroom compost which is divided into two phases: In phase I composting, bulk materials such as straw bedded horse manure, wheat straw, mulch hay, cotton or cocoa seed husks and gypsum are piled together, irrigated with water and consistently turned for a period of approximately two weeks. During this time, thermophilic microorganisms growing in the compost break down complex carbohydrates. A byproduct of this phase I composting is ammonia. This phase ends once the compost has reached a certain moisture content, has changed in color from a light brown to a dark brown due to caramelization reactions and has a strong ammonia odor. Once this is achieved, phase I compost is transferred into rooms where phase II composting begins. This phase consists of the pasteurization and conditioning of the compost. In order to obtain this, temperatures in the compost are gradually raised using steam for approximately 3 days until reaching a peak heat of 60 °C°. This peak temperature is maintained for at least 2 hours. It is followed by a conditioning period of 5 days where temperatures in the compost are dropped and maintained at 48-49 °C. Lastly, the compost is cooled down until temperatures are at approximately 27 °C. The main objectives of phase II composting are the elimination of hazardous microorganisms in the compost which may compete with *A. bisporus* mycelium (pasteurization) and the elimination of the ammonia left over from the previous composting phase (conditioning), which could also be detrimental to *A. bisporus*. Temperatures however, are kept at a threshold that allows beneficial microorganisms to survive,

which the *A. bisporus* will utilize as a main protein source (Beyer, 2003; Pecchia & Beyer, 2013). During the cool down period, the mushroom compost may be particularly susceptible to the invasion by mushroom sciarid flies (Keil, 2002). These flies are not obligate fungivores, rather, are decomposers, and can survive on unspawned mushroom compost alone (compost that has not been seeded with *A. bisporus* mycelia) (Kielbasa & Snetsinger, 1981; O'Connor & Keil, 2005). They are attracted to the odors coming from the compost and may readily lay their eggs in the compost once it has cooled down. This is not the case with mushroom phorid flies, which are obligate fungivores (Kielbasa & Snetsinger, 1981; Tibbles et al.,2005) and seem to be attracted to mushroom compost that has been spawned (compost with actively growing *A. bisporus* mycelia), therefore, unspawned compost is not vulnerable to the invasion of phorids.

Some mushroom growing operations perform phase II composting in tunnels, if so, at the end of phase II they transfer the compost to the growing rooms in which the mushroom farming cycle will occur. In this case it is said that the farm fills phase II compost. Other growing operations perform phase II composting inside the same growing rooms in which the mushroom farming cycle will occur, in this case it is said that the farm fills phase I compost. After either form of phase II composting, the mushroom growing substrate is spawned or seeded with the *A. bisporus* mycelia. Sterilized grains that have been inoculated with *A. bisporus* mycelia are broadcast and mixed into the compost after which the compost bed is covered with plastic material to keep the moisture in the compost. The whole operation, termed '*spawning*', usually takes from two to four hours and it is a crucial part of mushroom integrated pest management because during this period of time mushroom farm doors are opened, exposing the young crop to the invasion of mushroom flies. Once spawning is finalized, the doors to the growing room are shut and the stage known as *spawn run* begins. During this 14-17 day stage, the *A. bisporus* mycelia actively grows through the compost until colonizing the substrate entirely (Beyer, 2003; Pecchia & Beyer, 2013). Mushroom sciarid flies are typically attracted to the mushroom compost during the first days of spawn run.

The compost becomes less attractive to these flies the more colonized it becomes with mushroom mycelia (Kielbasa & Snetsinger, 1981; Cantelo & San Antonio, 1982). Mushroom phorid flies however, seem to be more attracted to the mushroom compost after the first 5 days or so of spawn run, when there is more mushroom mycelia in the compost and the mycelial odors are stronger (Richardson & Hesling, 1978;Scheepmaker et al., 1996). Either way, spawn run is a crucial time to focus fly management efforts by maximizing fly exclusion (maintaining doors shut, covering any openings such as cracks in the walls through where flies may get in). During this time it is important to monitor fly populations with light traps in order to make pesticide application decisions (Keil, 2002).

Once spawn run is finalized, a casing material made out of sphagnum meat moss must be added to the surface of the compost in order to stimulate the formation of the mushroom rhizomorphs, or stems. This casing layer also provides a medium of support and humidity (due to its water holding capacity) for the growing mushroom fruiting bodies. The operation in which this layer is added to the compost is termed *casing.* During this operation, similar as to when the spawn is introduced into the growing room, the mushroom doors are opened for a period of 2-4 hours, to apply the casing layer to the mushroom beds. During this 2-4 hour period, the crop is once again exposed to invading mushroom flies. Once the casing layer is fully applied the doors of the growing room are shut. During this period watering of the casing layer is crucial in order to support the developing rhizomorphs. Once mushroom rhizomorphs have developed, mushroom primordia (the initial stage of the mushroom cap) begin to grow until becoming mature mushroom caps. Mushroom harvesting begins 15–21 days after casing (Beyer, 2003; Pecchia & Beyer, 2013).

Mushrooms are harvested over a 2–4 day period in a 7–10-day cycle called flushes or breaks. When mature mushrooms are picked, an inhibitor to mushroom development is removed and the next flush moves toward maturity. Timing of the breaks or flushes is managed by control of the watering, CO2, and temperatures. The first two flushes account for the majority of the total

yield, with the subsequent flushes tapering off to relatively low levels of production (Beyer, 2003). During the harvesting period, doors are constantly opened and if not remain open in order to remove the harvested mushrooms from the room. At harvesting, the first generation of phorid flies emerges from the compost in very large quantities, becoming a nuisance for the harvesters and a risk for the other crops in subsequent rooms (Rinker, 1982). Anecdotal evidence from growers suggests that at harvest emerging (sciarid and phorid) flies exit the growing room and invade subsequent mushroom crops that might be at initial stages of the process. During harvesting, mushroom growers concentrate their efforts in keeping the flies inside the growing room. That is, preventing them from invading other rooms. Windows and (wharf end) doors are covered with plastic sprayed with Tangle-Trap ® (a sticky adhesive) in an effort to capture flies looking to exit the rooms. In Pennsylvania, mushrooms are harvested by hand in order to ensure uniformity and quality of the picked mushroom. Thus, mushroom production is labor demanding and labor intensive (Pecchia & Beyer, 2013).

Finally, the last stage of the mushroom farming process is termed *cook out* or *steam off*. Once harvesting comes to an end, the crop is then terminated. Before removing the mushroom substrate from the growing room, the grower "pasteurizes" it with steam in order to eliminate any disease causing microorganisms and pests that could infest subsequent houses or subsequent crop. The steaming-off procedure is accomplished by maintaining a compost temperature of 140–150ºF (60–70ºC) for anywhere from 8 to 24 hours. The steam off process is crucial to mushroom integrated pest management. High temperatures assist to eliminate any flies remaining in the compost or in the growing room once harvesting is done. Mushroom compost that has been pasteurized is termed spent mushroom substrate (SMS), mushroom soil/mulch or mushroom compost. This compost should be removed from the farm to reduce the chances of contaminating the subsequent mushroom crops at the farm (Keil, 2002; Pecchia & Beyer, 2013).

The mushroom sciarid fly *Lycoriella ingenua*

The sciarid fungus gnat *Lycoriella ingenua* (Dufour 1839) (Diptera: Sciaridae) is best known as a cosmopolitan pest in commercial mushroom farming in North America. Attention drawn to this species in the scientific literature is due to its economic importance and damage potential as a pest. This fungus gnat, formerly known as *Lycoriella mali* (Wetzel at al., 1982) causes some of the most severe insect damage to cultivated white buttoon mushrooms, *A. bisporus* (J.E.Lange) Emil J. Imbach (Agaricales: Agaricaceae), in the United States (Cloonan et al., 2016) Scientific research has been focused mainly on the fly's life history and behavior in mushroom farming conditions. Studies describe *L.ingenua* life tables, nutrition preferences in laboratory settings and potential control methods for the mushroom farming industry.

Description and Biology

Sciarid mushroom flies are black in color, their body length is about 3–5 mm long with long antennae and gray wings held folded over the back. Females tend to be more abundant and larger than males. Female sciarids have a pointed abdomen, while males have prominent claspers on the end of their abdomen that are used in mating. Adult flies do not actively feed but may take in some water. The immature sciarids (larvae) are translucent, white, legless maggots that range in length from 1–8 mm. The head is large and dark with powerful chewing mouthparts that distinguish sciarid larvae from other insect larvae that might be found in mushroom production houses. The larvae are the feeding stage in the life cycle of this fly (Cliff, 2002).

Most studied genera in the Sciaridae family have soil dwelling larvae that feed on a wide range of decomposing organic matter and on the fungi that inhabit these soil ecosystems or colonizes leaf litter (Frouz, 1999). In addition to mushroom houses, *L. ingenua* inhabits humid

woody areas (Cliff, 2002) where it feeds on decomposing material. Sciarid flies are not dependent on *A. bisporus* mycelia for completing their development, hence they are attracted to unspawned or freshly spawned compost (Kielbasa & Snetsinger, 1981; Cantelo & San Antonio, 1982). They can invade mushroom growing rooms once the mushroom compost is cooling down after the pasteurization process and continue to heavily invade the growing rooms during the first days of spawn run. Once inside the rooms, females lay eggs in patches in the compost and one female can lay up to 200 eggs (Cliff, 2002). Hatching larvae feed on both the compost and the *Agaricus* mycelia. At 24 C°, the life cycle from egg to adult lasts approximately 21 days (Lewandowski, et al., 2004).

Males emerge one or two days before than the females, after which they hover over the bed waiting for emerging females to mate with. In the presence of a female, the male starts flapping his wings rapidly and moves towards the female arching his abdomen between his legs while opening and closing his claspers. He then grasps the tip of the female's genitalia with the claspers, then he pivots 180 degrees, positioning himself in the opposite direction of the female. The mean pre-copulatory time, reported by is 36.6 seconds, while the mean copulation time is 11.2 seconds. After copulation, the female has period of inactivity (in this period the spermatozoa might be going through an 'activation process in the females body (MacDonald et al., 1977).

Mushroom sciarid flies and the mushroom growing substrate (mushroom compost)

It is not known what specifically attracts the female sciarid fly to the mushroom compost. It is well established, however, that females are attracted to microbial compounds in the compost, since sterilized compost (devoid of microorganisms) is not attractive to the females (Snetzinger et al., 1993, Cloonan at al., 2016). It has also been established that the more the compost is colonized with *A. bisporus* mycelia, the less successful are the sciarid larvae in completing development

Cantelo & San Antonio, 1982). Unsuccessful development on fully grown compost has been attributed to oxalate crystals that the *Agaricus* mycelia forms once fully developed, which may provide a physical barrier for the larvae, resulting in the larvae not being able to feed (Binns, 1980). Despite this hypothesis, there is not yet a definitive explanation.

Cloonan et al. (2016) also showed that the female is attracted to mushroom compost substrate rather than the mushroom mycelia itself. In addition, through ovipositional preference assays, they found that females are highly attracted to mushroom compost when it is infested with the pathogenic fungus, *Trichoderma aggressivum* over the mushroom compost alone. This finding further elucidated which one among the many microorganisms in the compost is possibly attracting the female. In further experiments they found that attraction to unspawned compost is due to microbial volatiles, such as those of, *Scatylidium thermophilium*, *Penicillium citrinum* and *Trichoderma aggressivum*, the latter remained the most attractive to the female (Cloonan et al., 2016 b) .

Some studies evaluated the effect of different species and or strains of cultivated mushrooms on the development of *L. ingenua*. O'Conner and Keil (2005) found significant differences in the weight of both male and female flies collected from farms cultivating different species of edible mushrooms; Portabella (*Agaricus bisporus*) Oyster (*Pleurotus ostreatus*) and Shiitake (*Lentinula edodes*). Portabella mushrooms were found to produce heavier females and males. They also found a positive linear relationship between female weights and fecundity regardless of the host the females were reared on; as the weight of the female increased, so did the number of eggs.

The mushroom phorid fly *Megaselia halterata*

The mushroom phorid fly, *Megaselia halterata* (Wood) (Phoridae) is a key pest in mushroom farming in most parts of the world (Binns et al., 1979; Pfeil & Mumma, 1993 Barzegar., 2016). Studies of *M. halterata* have mainly been done in laboratory settings, focusing on life table characteristics and substrate preferences (oviposition, survival and feeding behavior). Few studies have been done in commercial mushroom farming conditions, those that are in the literature have described aspects of their population dynamics.

Description and Biology

These flies are small, 1⁄8 inch (2–3 mm) in length, with a humpback appearance and very small antennae. On mushroom farms, the life history begins with female phorids entering the growing rooms during spawn run (Cliff, 2002). Females are attracted to the odor of mushroom mycelia present in the compost during this phase of the crop (a 15 day period of active *A. bisporus* growth) (Kielbasa & Snetsinger, 1981). According to Hussey (1960) one female can lay approximately 40-60 eggs in areas of mushroom compost where there is fresh mycelia growth. At mushroom growing temperatures (25 C°), the eggs hatch 2-3 days after oviposition. Upon eclosion, larvae begin selectively feeding on the mushroom mycelia. Larvae undergo three instars which are completed in 10 days after hatching (Hussey, 1960). The pupal stage lasts 14 days. Pupae are yellowish, flattened and oval in shape and 2 mm long, at the fourth day of pupation, they develop respiratory horns. Pupae attach themselves to the compost and develop a protective membrane that covers the entirety of it. Adult phorid flies mate 24-48 hours after emergence and females have a 2-3 day pre-ovipositional period after mating (Rinker, 1982). Adult longevity is dependent on temperature and relative humidity. Longevity increases with the decrease of temperature. At 24 °C

and 16 °C the lifespan of the adult stage is 4.2 and 8.3 days, respectively. Independent of temperature, relative humidity below 25% dramatically shortens the lifespan of both sexes (Kline, 1971). Likewise, the developmental time from egg to adult is highly dependent on temperature. The average time from egg to adult at 16 and 24 °C is 51 and 37 days, respectively (Rinker, 1982).

The mushroom phorid fly is an obligate fungal feeder and hence the gravid females are highly attracted to mushroom compost with actively growing mushroom mycelia (Sheepmaker et al., 1996). It has been suggested that 1-octen-3-ol and 3-octanone, volatiles of full-grown compost, act as attractants to the fly, but this could not be demonstrated in bioassays (Pfeil & Mumma, 1993). In laboratory settings, females prefer mushroom compost that has been in spawn run for 7-8 days (Richardson & Hesling, 1978).

Mushroom phorid fly studies outside and around mushroom farms

Studies on the phorid fly life history have been limited to laboratory settings and or inside mushroom farms (in the context of the mushroom growing cycle). Little is known about the ecology and behaviour of *M. halterata* outside of the mushroom farms or in other natural areas. Hussey, (1965) monitored *M. halterata* outside mushroom farms in the UK with suction traps and concluded that their flight is governed by both temperature and daylight. The author established a critical threshold for flight at 12.8 °C (yet stated that flight does not become general until the air reaches a temperature of 15.5 °C), even if in the morning hours when daylight is present, the phorid fly will not be active if temperatures are below the 12.8 °C temperature threshold. In the evenings, flight is curtailed by sunset, even when the temperature may be above the threshold for flight. The authors also found a negative correlation between wind velocity and phorid fly catches, as well as a positive correlation between daylight and phorid fly catches.

Results from Hussey's experiments (1965), done on mushroom phorid flies caught on traps outside spawning rooms, showed a female to male sex ratio of 1:62 in the traps. The latter led the author to set up traps outside mushroom farms (near a large dump of spent compost, in a hedgerow 50 meters from the nearest mushroom house and at two sites within a few yards of cropping houses on two mushroom farms), where all together the female to male sex ratio was once again male biased; 1:27.

Hussey (1965) reported aggregations *of M. halterata* in sites unassociated with mushroom farms: In and outside the walls of an office building and in a bungalow at least ¾ of a mile from a mushroom shed. In both instances the female to male ratio was male biased, 1:5.2 and 1:87, respectively .These observations of aggregations of *M. halterata* both near and at a distance from mushroom farms, along with the majority of male flies suggests that *M. halterata* may move from the site of maturation towards the outside of the farms and that females may move in response to stimuli from the males for mating and to the growing mycelium for ovopositing.

Richardson & Hesling (1977) wrote that phorid fly populations increased from spring to autumn and were rarely seen during the winter in England. Through controlled experiments they showed that adults are capable of overwintering from December to early May. Binns et al., (1979) monitored phorid fly populations on a mushroom farm and concluded that in England their peak activity was in August. Keil (2002) wrote that in North America phorids become a problem in mushroom farms in the months of June and July.

Population dynamics of M. halterata within commercial mushroom crops

Mushroom phorid fly population dynamics have been reported in the literature through three per reviewed papers and one doctoral dissertation. Hussey (1965) monitored phorid fly adults populations in commercial mushroom houses with the use of light traps and found that *M. halterata*

numbers increased from day seven to day 14 of spawning, meaning that females were likely attracted to one week old mycelium. In the same study phorid fly catches also increased until the first week of casing. A second generation of flies, (progeny of those eggs laid at casing) would appear in the fifth week of cropping. The author also determined that the female to male sex ratio of phorid flies caught on the traps was 1:1.06, quite different from those sex ratios found outside the farms (Hussey 1965).

Bins et al (1979) monitored *M. halterata* populations in commercial mushroom farms consisting of separate spawn run and cropping rooms (the spawn run stage is carried out in one room and then the same compost is moved to another room where the casing and cropping stages are carried out). The author describes that initial *M. halterata* populations entered the spawn run rooms from day 2 to day 15 of spawn run. Afterwards, in the cropping rooms, fly catches increased from the application of the casing layer to crop cook out, approximately 10 weeks later. The author attributed these catches to the emerging first generation of flies, progeny of the flies entering the spawn run rooms. The same study reported that while during the spring and fall fly numbers between spawn and cropping rooms were related, during the cold winter months, there was a clear maintenance of fly populations in the cropping houses, when flies were nearly absent in the spawn running rooms. The authors suggested an independent and, possibly, non-reproducing cropping-room population in the closed unit during the winter. In addition, the examination of mushroom beds in these cropping rooms revealed that dry debris held large numbers of immobile, adult phorids, suggesting an ability to hibernate in mushroom houses during cold winter months. The mechanisms of the overwintering mushroom phorid fly is still yet to be studied.

Navarro et al. (2001) found that *M. halterata* adults infested mushrooms later in the production cycle and were affected by compost temperatures. They monitored phorid fly populations within mushroom farms with the use of light traps over 4 seasons (one year). In their experiments, phorid flies were very low during the spawn run phase, but a peak was seen during

casehold. The authors attributed these peaks to the flies entering the mushroom houses when doors are opened for the application of the casing layer. A significant second peak in fly catches occurred during first break showing the emergence of the first generation proceeding from initial fly infestations. The authors also report that the emergence of the first generation of phorids was affected by the temperature of the compost. At compost temperatures of 22 °C during the summer, the first generation of mushroom flies emerged during first break. At compost temperatures of 20 °C in the Spring, emergence of the first generation was at third break. Fly traps placed at the rear end of the mushroom house as opposed to the middle and entrance, caught more phorid flies, the authors attributed this finding the vents and exhaust windows located at the rear end, being a possible entryway for the flies into the house (Navarro et al., 2001).

Jess et al. (2007) studied phorid fly activity on mushroom compost wharfs and found close to no activity of phorid flies on phase I and II composting areas, neither at the bagging area, where compost is placed into bags before being transported to the farms. They concluded that phase II mushroom compost is not a source of fly introduction.

Mushroom phorid flies are often seen in what appears to be swarms outside of mushroom farms (personal communication), a behaviour that has not been reported in the literature. My own preliminary sampling of these swarms with the use of nets has shown phorid fly adults in copula while flying. To that regard, Hussey & Gurney (1964) reported that a period of flight is required for *M. halterata* before mating. Mating swarms are a common behavior in other dipteran species, and are often characterized by a quasi-stationary flight over a landmark in which mating takes place (Downes, 1968).

The mushroom phorid fly damage to the mushroom crop

Mushroom phorid fly larvae are not capable of causing the kind of damage that sciarid fly larvae cause. Significantly more phorid larvae can be tolerated—perhaps as much as 50 to 100 times more than sciarids before economic damage can occur to the crop. Although large numbers of phorid larvae are needed to cause significant yield losses, adult flies are a nuisance to growers. The adults also act as mechanical vectors of fungal diseases such as dry bubble disease caused by *Lecanicillum fungicola* (White, 1986) and there is anecdotal evidence that they also vector *Trichoderma aggressivum*, causal agent of green mold disease in mushroom farming. Phorids also cause nuisance to residential areas near farms, when they enter private homes (Hussey, 1965).

Mushroom flies and green mold disease

One of the major threats to mushroom farming worldwide is the potentially devastating green mold disease, caused by the fungal pathogen *Trichoderma aggressivum* ft *aggressivum Samuels & Gams* (North America) and *Trichoderma aggressivum* ft *europaeum* Samuels & Gams (Europe) (Savoie at al., 2001; Guthrie & Castle, 2006). These two aggressive strains are genetically distinct yet closely related, formerly named *T. harzianum* Th2 and Th4, respectively, are endemic to mushroom farms (Coles & Barber, 2002).

Green mold appeared in Pennsylvania, USA, in the early 1990s in cultivated mushroom beds. Since the onset of the disease in this state, crop losses have been estimated in excess of 30 million (U.S) dollars (White et al., 1996).

The disease is characterized by a dense gray hyphal growth on mushroom compost or casing materials, that later changes to white, followed by sporulation that is dark green color. There is an almost complete lack of mushroom production in infected areas (Coles & Barber, 2002;

Guthrie & Castle, 2006). Many factors have been associated with the occurrence of green mold disease such as the nature of raw materials and composting procedures, reduced attention to sanitation and post crop steam off programs (Anderson et al., 2001). However, no specific compost or environmental conditions have been found to be associated consistently with green mold development. Spores are easily carried by workers and contaminated equipment. Spores can also be carried into the growing rooms through substrate materials that have not been properly composted or unpasteurized materials from harvesting such as mushroom trimmings (Coles & Barber,2002).

The control of Green Mold disease relies on prevention. Measures include strict sanitation protocols such as disinfecting farm equipment, farm worker shoes prior entering the mushroom houses and timely removal of any potential inoculum source from the farm. Proper composting procedures are crucial in preventing the disease. During compost preparation materials should be cross mixed to ensure exposure of all the material to high temperatures. Ensuring high temperatures (60 C) for at least two hours during pasteurization of phase II composting can kill any green mold spores present in the compost. Steaming the compost at the end of the crop, before it is taken out of the growing room prevents the spread of green mold spores to other areas of the farm (Pecchia & Beyer, 2013). Inside the mushroom growing rooms a control tactic is to apply salt over patches of the mushroom compost where green mold has formed (Anderson 2001; Coles & Barber 2002). When *T. aggressivum* has spread considerably in a growing room the common protocol is for the crop to be destroyed with steam and emptied immediately to prevent the infection of other crops (Fletcher and Gaze 2008).

Mushroom flies, such as *Lycoriella ingenua* Dufour (Diptera: Sciaridae) and *Megaselia halterata* (Diptera: Phoridae), both pests in mushroom farming, are conjectured to spread green mold disease because *T. aggressivum* spores are contained in a sticky matrix that can easily attach to the fly bodies (Anderson et al., 2001; Coles and Barber 2002). The flies can acquire the spores

when they land on *T. aggressivum*-infested compost patches, and then spread the spores to other areas of the compost bed through further movement. However, no definitive studies have been done, but there tends to be a positive association between fly populations and green mold disease incidence in mushroom farming houses.

Studies have shown that the severity of green mold disease is related to the time between the initial pathogen infestation and green mold appearance, more significant yield losses occurred when green mold sporulation is detected early in the crop cycle (Anderson et al., 2001). Given that *L. ingenua* enters growing rooms at an early stage of the crop and its attraction to compost containing *T. aggressivum* spores (Cloonan et al., 2016), there is a risk of a spread of the spores to other areas of the compost as the females move while ovipositing and spreading the disease at the most susceptible stage of the crop.

External acquisition (onto the exoskeleton) and dispersal (vectoring) of fungal spores onto crops by dipterans has been documented in many insects such as shore flies (Ephrididae), moth flies (Psychodidae) fungus gnats (Sciaridae). Adults from the genera *Bradysia* and *Scatella* can physically acquire spores of soil borne fungal pathogens such as *Fusarium acuminatum* and *Fusarium oxysporum* from infected greenhouse plants and subsequently infect healthy ones (El-Hamalawi, 2008; Scarlett, et al., 2014). Other studies have also described how sciarid larvae larvae can acquire fungal propagules by ingestion and excrete viable spores through frass deposits, causing disease spread to seedlings in nurseries (Gardiner et al., 1990; Stanghellini et al., 1996; Braun et al., 2012)

Although Shamshad et al. (2008) documented the vectoring of another recurring disease in mushroom farming (dry bubble disease caused by *Verticillum fungicola*) by *L. ingenua*, no quantitative data exists regarding the role of *L. ingenua* as a vector of *T. aggressivum.*

Hispanic farmworkers and the Health Belief Model

Results from the last National Agricultural Workers Survey (NAWS) showed that eighty percent of all farmworkers in the US were Hispanic. Sixty-eight percent of hired farmworkers interviewed in fiscal years 2013-2014 were born in Mexico and even among U.S.-born workers, 27 percent were Hispanic. Each year, approximately 45,000 to 50,000 migrant and seasonal farm workers are employed in Pennsylvania to assist in harvesting the Commonwealth's fruit, vegetable, and mushroom crops (Carson et al., 2004).

Farmworkers face great exposure to pesticides applied during the growing, harvesting transporting and processing of food (Halfacre-Hitchcock et al., 2006). The US Environmental Protection Agency designed the Worker protection Standard which requires that all farmworkers be taught the dangers of chemical exposure and how to protect themselves from chemical exposure (Quandt et al., 1998). The cultural and educational appropriateness of these regulations has been questioned given that studies have found that even though farmworkers are given information about the risks of pesticide exposure and how to protect themselves from it, they fail to perform pesticide safety behaviors (Vaughan, 1993; Quandt et al., 1998; Austin et al., 2001; Arcury et al., 2002; Cabrera & Leckie, 2009).

A common model utilized for studying pesticide safety behaviors among Hispanic farmworkers is the Health belief Model (HBM) (Rosenstock, 1966; Rosenstock et al., 1988) which seeks to assess how behavior is a function of a person's subjective appraisal of risk. The HBM proposes six key concepts that determine whether a person will engage in a risk mitigating behaviour:

1. Perceived susceptibility: An individual's belief that he/she is at risk of an outcome.
2. Perceived severity: By how much the risk is or how severe the individual believes the consequences of the risk are.

3. Perceived Benefits/barriers of a risk (mitigating) behaviour: The belief that a behavior will modify the outcome.
4. Self-efficacy: The belief that one can or cannot take action to mitigate or modify the outcome.
5. Cues to action: Knowledge provided by experience, educational material, training etc.
6. Demographic characteristics: Age, ethnicity, socioeconomic status, etc.

According to the HBM, persons must perceive themselves susceptible to risk before they will take action. The relationship between risk and health action is modified by self-efficacy that is, recognizing one's ability to control exposure to harm (Arcury et al., 2002).

Studies on pesticide safety conducted with Hispanic farmworker populations often simplify the HBM model, exploring the correlation between certain variables or the effect of a single or set of variables on the behavioural outcome (a risk mitigating behaviour). For example, Arcury et al (2002) studied how safety information and farmworker knowledge (cues to action) affect pesticide risk (risk susceptibility and severity) and control (self-efficacy) perceptions and ultimately, safety behaviors among farmworkers in North Carolina. Results showed that receiving safety information and having knowledge on safety increased the perception of having control over safety issues in the workplace. Likewise, engaging in safety behaviors was also strongly related to perceptions of control. However, even though knowledge on pesticide exposure was strongly related to perceived risk of pesticide exposure, neither was related to safety behavior. The authors concluded that for pesticide safety education to be effective, it must address issues of farmworker control in implementing workplace pesticide safety. Another study with a group of Hispanic farmworkers in California, (Cabrera & Leckie, 2009) found that the community, despite having high risk perceptions on pesticide exposure and having received information on the health effects of pesticide exposure, continued to engage in risky behaviors.

Perhaps the most studied variables of the HBM are risk and control perceptions, which have a cultural and social basis (Bradbury, 1989; Covello & Johnson, 1987; Dietz et al., 1989; Nelkin, 1989) and are conceptualized as embedded in complex systems of beliefs, values, and prior experiences, likely to vary among different groups in society (Bradbury, 1989; Douglas & Wildavsky, 1982; Nelkin, 1989; Vaughan & Nordenstam, 1991). Studies among Hispanic farmworkers have revealed interesting cultural beliefs influencing risk and control perceptions among this population. For example, a recurrent belief reported in the literature is that that susceptibility to the effects of pesticides is highly individualized; some persons are sensitive and experience ill effects, and others are inherently more resistant (Vaughan, 1993; Quandt et al., 1998) . Snipes et al (2009) found that farmworkers believed that men were less likely to be negatively affected by pesticide exposure than women. Likewise, risk was believed to be larger for individuals with a 'weak body type'. While studying perceived control, Grieshop et al (1996) found that California farmworkers attributed more control over workplace safety to factors outside of themselves (e.g., in God, luck, or supervisors) than to factors they could control. In farmworker populations, perceptions of control and the practice of risk mitigating behaviors are often related to financial issues. For example, using protective equipment (a risk mitigating behavior) may slow down their work, reducing the amount of produce they pick and hence their pay (Snipes et al., 2009).

The HBM is a useful conceptual starting point for understanding risk behaviour and in the case of pesticide safety, results from applying this model have implications for the education and training of Hispanic farmworkers about pesticide safety. For example, issues of farmworker control in implementing workplace pesticide safety must be addressed. Farmworkers' pesticide-relevant beliefs regarding perceived danger and susceptibility to pesticides should be taken into consideration when designing and implementing safety interventions (Vaughan, 1993;Quandt et al., 1998; Quandt et al., 2001;Arcury et al., 2002; Cabrera & Leckie, 2009; Snipes et al., 2009).

The HBM has not yet been applied to the context of Integrated Pest Management implementation or 'IPM behavior' among farmers and farmworkers. Research regarding farmers' perceptions of pests and IPM has mainly focused on farmers' knowledge of IPM, perceived risks of pests and pathogens and perceived feasibility (perceived barriers) of applying IPM (Papadaki-Klavdianou et al., 2000; Nyeko et al., 2007; Balogun & Musa, 2013; Cockburn et al., 2014; Midega et al., 2016). The HBM incorporates issues of control (or self-efficacy) and demographic characteristics that may help explain risk perceptions as well as control perceptions and the effect of these variables have on behavior.

In some crops, the success of IPM relies on the extent and consistency with which it is implemented by farmers and farmworkers alike (Pecchia & Beyer, 2013). In these cases, risk perception studies must include farmworkers. In addition, farmworkers are constantly exposed to pesticides and the implementation of IPM can be one of tools for minimizing the levels of pesticide exposure through the reduced use of pesticides as a result of a successful IPM program.

Chapter 2

The mushroom sciarid fly, *Lycoriella ingenua*, benefits from its association with green mold disease (*Trichoderma aggressivum*) in commercial mushroom production

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Abstract

The mushroom fly, *Lycoriella ingenua* Dufour (Diptera: Sciaridae), is a pest in white button mushroom (*Agaricus bisporus)* farming in North America. The main risk associated with sciarid flies inside mushroom farms is that the adult can potentially vector mushroom green mold disease caused by the pathogenic fungus *Trichoderma aggressivum* Samuels & W Gams (Hypocreales). Flies are attracted to *T. aggressivum* infected compost, and through subsequent movement are suspected to spread the spores. The present study evaluated whether there is a fitness benefit for the sciarid flies from their association with *T. aggressivum*. *Lycoriella ingenua* was reared on three substrates: 1) spawned mushroom compost inoculated with the *T. aggressivum,* 2) spawned mushroom compost and 3) unspawned mushroom compost. Developmental time from larva to adult, adult longevity, adult fecundity and female body size were used as indicators of fly fitness. There was a fitness benefit for the sciarid fly when larvae develop on spawned mushroom

compost parasitized by green mold, including higher adult emergence rate, faster development time from larva to adult and larger adult females. Fly fitness declined when the compost was fully colonized by *A. bisporus* mycelia and *T. aggressivum* was not present. This suggests that sciarid larvae benefit from the *T. aggressivum* parasitism on *A. bisporus* and the green mold. Benefits may include improved nutrition, defense suppression or pre-digestion.

Introduction

The mushroom fly *Lycoriella ingenua* Dufour (Diptera: Sciaridae) is a pest in white button mushroom (*Agaricus bisporus)* farming in North America (Wetzel et al. 1982). Female flies invade mushroom production houses as soon as the compost substrate begins to cool down after a pasteurization process (Phase II compost) and mesophilic fungi in the compost begin to grow (Keil 2002). Cloonan et al. 2016 demonstrated that females are specifically attracted to the microbial activity characteristic of Phase II compost. One female can lay up to 150 eggs (Keil 2002), and larvae can mechanically damage the compost and the mushroom mycelia through feeding. However, significant yield losses are only observed when large numbers of sciarid larvae are present (White 1986). The first generation of adult flies normally emerges before the first crop of mushrooms is picked (approximately eight weeks after Phase II) and continues to emerge during cropping. Newly emerged adults mate and lay eggs in the compost to continue the infestation cycle. Populations grow exponentially and can become very numerous inside the growing rooms (Keil 2002).

The main risk associated with sciarid flies inside the mushroom growing rooms is the adult's potential vectoring of fungal disease spores (Coles and Barber 2002; Keil 2002; Shamshad et al. 2009) including those of the potentially devastating mushroom green mold disease (Anderson et al. 2001; Guthrie and Castle 2006) caused by the pathogenic fungus *Trichoderma aggressivum*

Samuels & W Gams (Hypocreales). The colonization mechanisms of *T. aggressivum* in mushroom compost are not fully understood, but studies *in vitro* suggest that they involve both mycoparasitic and saprotrophic components (Williams et al. 2003). Green mold disease is characterized by a dense hyphal growth in the compost and casing layer. In later stages of the infestation sporulation occurs and green spores become visible on the surface of the mushroom bed (Anderson et al. 2001; Guthrie and Castle 2006). In areas colonized by *T. aggressivum*, mushroom fruiting body formation is retarded and those that do form may be of poor quality (Largeteau and Savoie 2010). Sciarid fly populations have been suspected in the spread of green mold disease because *T. aggressivum* spores are contained in a sticky matrix that can easily attach to the fly bodies. The flies can acquire the spores when they land on *T. aggressivum* infested compost patches, and then spread the spores to other areas of the compost bed through further movement (Anderson et al. 2001; Coles and Barber 2002). When given a choice, *L. ingenua* females have an ovipostion preference for mushroom compost infested with *T. aggressivum* compared to un-infested compost (Cloonan et al. 2016).

Since flies are differentially attracted to infested compost, we explored whether the flies may benefit from their association with *T. aggressivum*. The objective of this study was to determine fitness differences between flies reared on *T. aggressivum*-infected spawned mushroom compost versus spawned compost without *T. aggressivum*.

Materials and methods

Fly rearing

The flies used for this experiment were from a 4-year-old laboratory colony maintained at the University Park Campus of Pennsylvania State University, Department of Entomology. The flies in the laboratory colony were reared on a mixture of phase II mushroom compost with a

nitrogen supplement (100:1, w/w) in an environmental growth chamber at 21°C, 70% r.h., and L12:D12 photoperiod.

Substrates

Unspawned mushroom compost

Compost was prepared at the Mushroom Research Center (MRC) at the Pennsylvania State University. Bulk ingredients used were wheat straw-bedded horse manure, dehydrated poultry manure, distiller's grain and gypsum. These ingredients approximate those used in commercial mushroom production practices. Raw materials were mixed with water in a Jaylor® feed mixer (Jaylor Fabricating 071213 10th Line East Garafraxa, Ontario L9W6Z9, Canada) to a starting moisture of 60%. This mixture was placed into an aerated bunker for three days. Air was supplied to the mix in order to prevent anaerobic conditions. Compost temperatures reached approximately 80 °C during the first three days of Phase I composting. On day 4, the material was removed from the bunker, placed in the feed mixer with the addition of supplemental water and again placed into the aerated bunker for three more days with temperatures again reaching approximately 80°C. Three days afterwards, the material was removed from the bunker, mixed with additional water and placed as an approximately 15 cm deep in 1.5 m^2 wooden trays. The trays were placed into an environmentally controlled room for an 8-day Phase II composting period. Pasteurization was achieved at 60-65°C for 2 h, followed by conditioning in which the compost was held at 48°C to eliminate free ammonia. Finally, the compost was cooled to a temperature below 28ºC.

Spawned mushroom compost

Phase II mushroom compost (described above) was weighed into 22.7 kg (50 lb) capacity tubs. For ease of mixing and incorporation of the spawn and supplement, two tubs were each filled with 11.3 kg (25 lb) phase II compost. Commercial, off-white hybrid *A. bisporus* millet spawn was added at a rate of 100 g per 11.3 kg compost, along with 102 g of a commercial, delayed-release supplement (a proprietary blend of plant-based lipids, carbohydrates and protein, manufacturer Promycel Gold). The compost was then thoroughly mixed by hand until the components were evenly distributed throughout the compost. The content of the two 11.3 kg tubs were then combined into a single tub and compressed using a homemade hydraulic press.

Inoculation of spawned mushroom compost with T. aggressivum

A pure culture of *T. aggressivum* f. *aggressivum* (#324; Chester County Farm; Romaine) was obtained from the Department of Plant Pathology and Environmental Microbiology (Pennsylvania State University). This culture was used to inoculate three 9 mm diameter, malt agar plates. The inoculated plates were incubated at 25°C for five days to allow the fungal culture to colonize the agar and sporulate. A spore suspension was made, by pipetting 10 mL of sterile 0.05% Tween 80 solution onto one of the colonized plates. The fungal conidia were dislodged with a pipette and poured into a bag containing 50 g of sterilized barley grains. *Trichoderma aggressivum* mycelia began to colonize the barley grains after 3 to 5 days incubation at 25°C. The infested barley grains were then transferred and mixed thoroughly into 500 g spawned compost in a sterile bag, which was placed into an incubator at 25ºC. The use of the grains was solely to encourage the growth of *T.aggressivum*, hence we used a low barley to substrate ratio (1:11) that would not create bias in the treatment. Likewise, we waited an additional 5 days for the fungus to completely degrade

the barley and colonize the compost before its use in the experiment. The result was a highly decomposed spawned compost, similar to a *T. aggressivum* infested compost found in commercial mushroom conditions.

Experimental design

Lycoriella ingenua were reared on three different substrates: 1) fully spawned (with *A. bisporus*) mushroom compost inoculated with *T. aggressivum,* 2) fully spawned (with *A. bisporus*) mushroom compost and 3) unspawned (phase II) mushroom compost. Since sciarid flies develop successfully in unspawned mushroom compost, this was used as a control. We measured developmental time from larva to adult, female adult fecundity and female body size as indicators of fly fitness.

The experiment was conducted in an environmental growth chamber at 21°C, 70% r.h., and L12:D12 photoperiod. Newly emerged and mated females from the colony were aspirated from the rearing cages [white popup cages with a single vinyl window (30 x 30 x 30 cm; Raising Butterflies, UT, USA)] and placed onto 60 x 15 mm polystyrene Petri dishes (Crystalgen, Inc., NY, USA) containing approximately 7 ml water agar (15 g L^{-1} agar) for oviposition. The Petri dishes containing the eggs were placed in a growth chamber (same conditions as above) and the eggs were monitored until larval eclosion, which occurred approximately five days later.

Newly emerged (<24 hr) larvae from the petri dishes were used for the experiment. Using a size 1 paint brush (Artist's LoftTM, MSPCI, TX, USA), single larvae were taken from the petri dish and placed individually 30-ml (1oz.) plastic portion cups (4 cm diameter, 3 cm high; Dart Solo, Harrisburg, PA, USA) fitted with a snap-top plastic lid and containing 10 g of one of the three substrates. One hundred larvae were used per substrate. The portion cups containing the substrate

and individual 1-day-old larvae were placed randomly in the environmental growth chamber. These cups were monitored on a daily basis for adult emergence.

Immediately after emergence, adults were sexed based on morphological characteristics (male adults have a pair of claspers on the last abdominal segment) (Lewandowski et al. 2004). Within each treatment (substrate), pairs of adults were placed into 10 ml disposable culture tubes (15 × 85 mm; VWR International, PA, USA) covered with Parafilm M (Bemis Healthcare Packaging, WI, USA) for mating. After visual confirmation that mating had occurred, the pairs were separated. Males were placed into clear plastic containers (11.5 cm diameter, 7.5 cm high; Dart Solo, Harrisburg, PA, USA). Females were placed into the same type of plastic container, which also contained a water agar petri dish as an oviposition receptacle. Both sexes were monitored in their separate containers until death.

Once the egg laying females died, the water agar petri dishes were removed from the cups and the eggs were counted using a microscope (Olympus SZ61, Hunt Optics & Imaging Inc. Pittsburgh, PA). The number of eggs was recorded for each ovipositing female. The wing length (distance in mm from the fore wing insertion on the mesothorax to the wing apex denoted by R5) and body length (distance in mm from the cephalic vertex to the tip of the gonostyle) for each ovpositing female were measured by placing the fly specimen over a millimetric scale and viewed through the microscope ocular. The entire experiment was then repeated.

Statistical analysis

The effect of substrate on adult emergence was analyzed with a Chi Square Analysis followed by a pairwise comparison test with a Bonferroni Correction of the *P* value (0.05) (McDonald 2014). The effect of substrate on male, female and overall developmental time, fecundity, female wing length and female body length, were analyzed using the General Linear

Model test in SPSS v.23 (p = 0.05) (IBM 2015). The effect of experimental repetition was not significant in this model, therefore the data were pooled for analysis. Means were separated using the Tukey multiple comparisons test. All measures of variation around the means reported are standard errors.

Results

Emergence

The type of substrate had a significant effect on adult emergence (χ^2= 33.667, d.f. = 2, P < 0.001) (Fig. 2-1). Pairwise comparisons indicated that emergence of adults from spawned compost inoculated with *T. aggressivum* and unspawned compost (156 and 143 adults emerged, respectively) was significantly higher than from the spawned compost (83 adults emerged) (Fig.2).

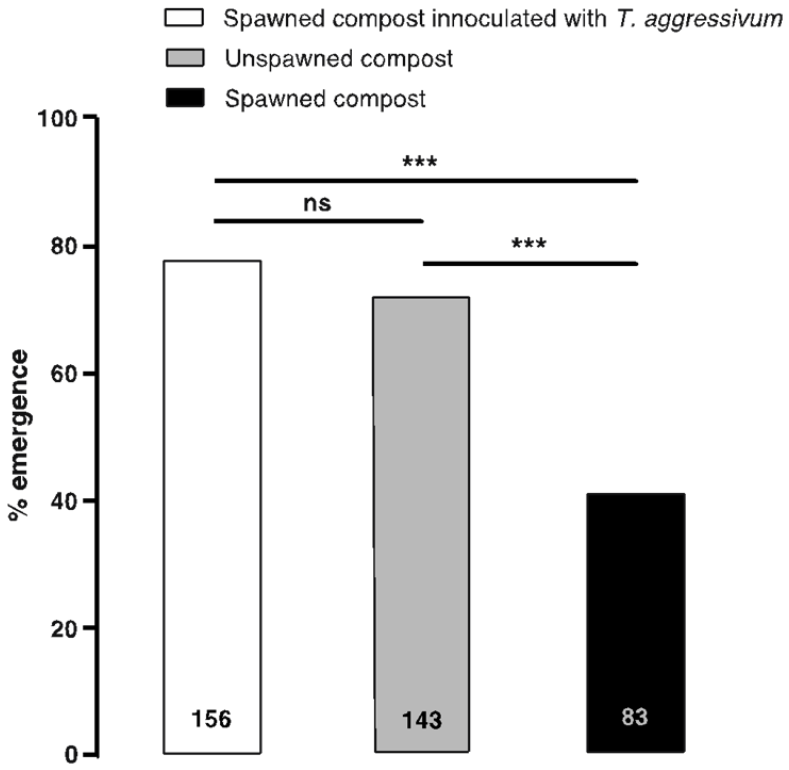

Figure 1: Percentage of *L. ingenua* emerged from *T. aggressivum* inoculated spawned compost, spawned compost and unspawned compost. Numbers in the bars indicate the number of neonate larvae that developed to adults on each substrate. Initial number of neonate larvae used on each substrate (n=200). Statistical significance is indicated by *** ($P < 0.001$) and ns ($P > 0.05$, not significant) (X^2 test; pairwise comparisons with a Bonferroni correction; $a = 0.016$)

Development

There was a substrate effect on male developmental time ($F_{2, 181} = 72.512$, $P < 0.001$) (Fig. 1). The shortest developmental time was observed on the spawned compost inoculated with *T. aggressivum* (18.1 ± 0.2 days, n= 87), followed by the unspawned compost (19.6 ± 0.2 days,

n=62). The longest male developmental time was observed on the *A. bisporus* fully spawned compost (23.3 ± 0.5 days, n= 37) (Fig. 2).

Likewise, substrate type affected female development time ($F_{2, 184}$ = 37.099, P < 0.001) (Fig. 2). Females developed significantly faster on the spawned compost inoculated with *T. aggressivum* (20.0 ± 0.3 days, n = 73) than on the spawned compost (23.8 ± 0.5 days, n =85). There was no significant difference between the developmental time of female flies reared on the *T. aggressivum* inoculated spawned compost and the unspawned compost (20.6 ± 0.2 days, n = 42) (Fig. 2).

There was an effect of susbrate type on overall (male and female) developmental time ($F_{2,368}$ = 95.750, P < 0.001) (Fig. 2). The shortest developmental time was observed on the spawned compost inoculated with *T. aggressivum* (19.0 ± 0.2 days, n= 156), followed by development on unspawned compost (20.2 ± 0.1 days, n = 143). *Lycoriella ingenua* larvae took the longest time to develop to the adult stage when reared on the spawned compost (23.5 ± 0.4 days, n = 83) (Fig.2).

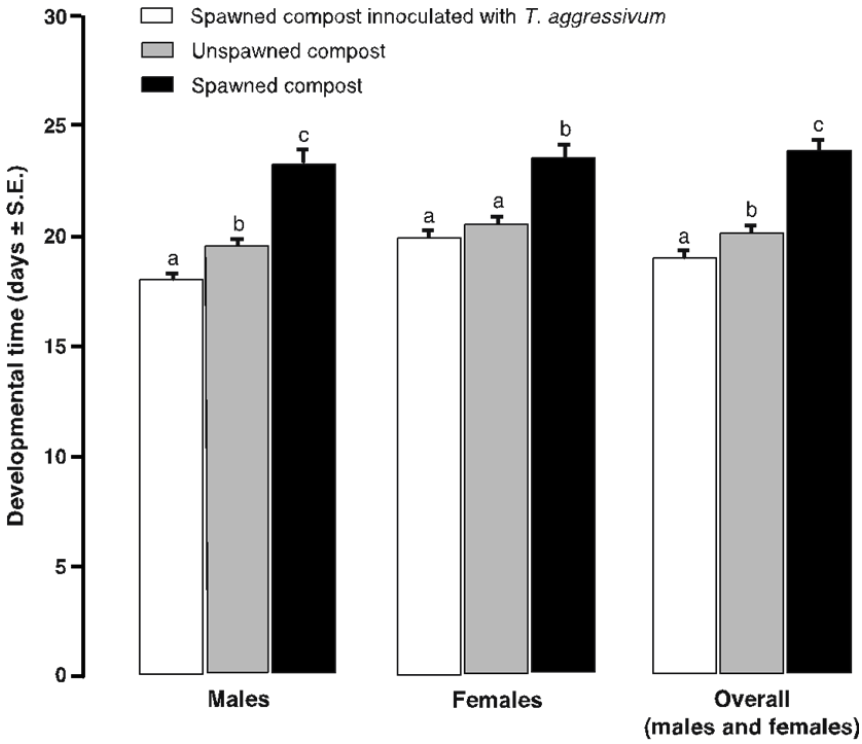

Figure 2: *Lycoriella ingenua* male, female and overall (both males and females) developmental times reared on spawned compost inoculated with *T. aggressivum*, unspawned compost and spawned compost. Developmental times are measured as number of days from placement of neonate larvae to adult emergence within sex among substrates. Bars with different lettering denote significant differences among means within sex (GLM with poisson error distribution followed by Tukey's HSD, $P < 0.001$). Error bars represent SEM.

Female wing length

Substrate had a significant effect on female wing length ($F_{2, 91} = 3.720$, $P = 0.028$) (Fig. 3). Gravid females that emerged from spawned compost inoculated with *T. aggressivum* had a significantly larger wing length (3.2 ± 0.04 mm, n=73) than those that emerged from the spawned compost (3.0 ± 0.04 mm, n = 85). Females that emerged from the unspawned compost (3.1 ± 0.05

mm, n= 42) also had a larger wing length than those that emerged from and the spawned compost. No significant differences in wing length were observed among females that emerged from spawned compost inoculated with *T. aggressivum* and unspawned compost (Fig.3).

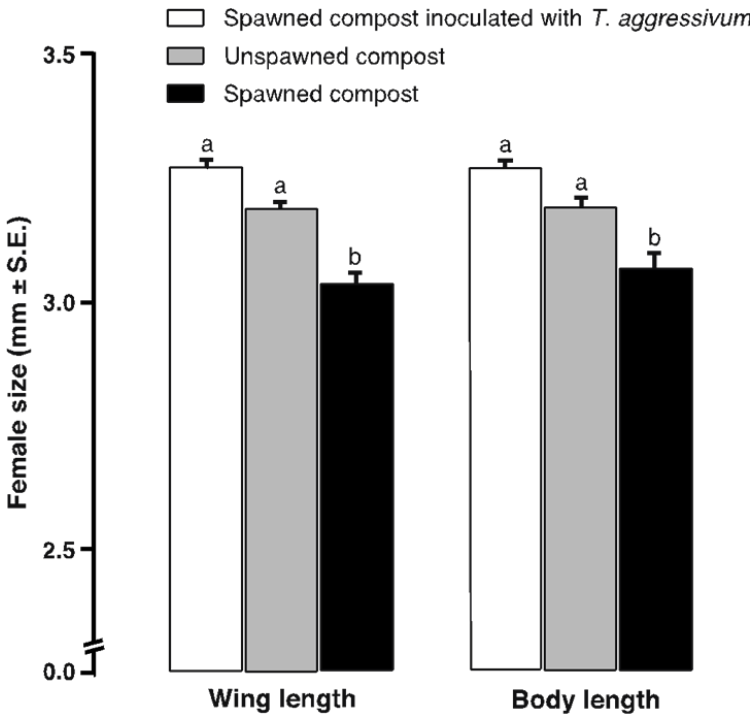

Figure 3: Wing and body length measurements of gravid *L. ingenua* females that emerged from spawned compost inoculated with *T. aggressivum*, unspawned compost and spawned compost. Bars with different lettering denote significant differences among means within substrates (GLM with poisson error distribution followed by Tukey's HSD, $P < 0.001$). Error bars represent standard errors of the means.

Female body length

Substrate had a significant effect on female body length ($F_{2, 89} = 6.392$, $P = 0.003$) (Fig. 3). Females that emerged from spawned compost inoculated with *T. aggressivum* were significantly larger (3.2 ± 0.14 mm n = 73) than those that emerged from the spawned compost (3.0 ± 0.31 mm n= 85). Females that emerged from the unspawned compost (3.2 ± 0.20 mm n = 42) were also significantly larger than those that emerged from spawned compost. No significant differences in body length were observed among females that emerged from spawned compost inoculated with *T. aggressivum* and unspawned compost (Fig. 3-3).

Discussion

Results show that there is a fitness benefit for the sciarid fly when fully spawned mushroom compost is infected with green mold, represented in a higher adult emergence rate, a faster development time from larvae to adult and larger sized adult females. Conversely, fully spawned compost without *T. aggressivum* had a negative effect on the sciarid fly. Emergence was significantly lower, developmental time for both males and adults was significantly longer and adult females where significantly smaller in size.

Various mechanisms (non-mutually exclusive) may explain our results. (1) In a nutritional sense, compost that is fully colonized with *A. bisporus* mycelia may be lacking the necessary nutrients for sciarid larvae development. *Trichoderma. aggressivum* mycelia itself or the products of metabolism may enhance the nutritional quality of a nutrient poor (spawned) compost. (2) In an antagonistic scenario a fully developed *A. bisporus*, may be suppressing larval development through defense mechanisms. When antagonized by *T. aggressivum*, those defense mechanisms against the sciarid larvae may be hampered, resulting in an improvement in larval development.

The rate of *A. bisporus* growth in the compost has a negative effect on sciarid emergence. The more mushroom mycelium that is present in the compost, the fewer adult sciarids emerge (Cantelo and San Antonio 1982; Smith et al. 2006). The latter author attributed their findings to competition between *L. ingenua* and *A. bisporus* for nutrients in the compost, while the second attributed results to both the extent of fungal growth in the compost and fungal isolate strain. We found negative effects of *A. bisporus* mycelial growth on other life history traits as well. This suggests that the more colonized the compost with mushroom mycelia, the less suitable it becomes for sciarid larval development. *Agaricus bisporus* utilizes the microbial community present in the compost as a (protein) food source (Sparling 1982). Beneficial microbe conservation is the reason that mushroom compost is pasteurized rather than sterilized. In our study, unspawned compost was a suitable substrate for sciarid development and given that sterilized compost (lacking microbes) is unsuitable for *L.ingenua* larval development, the microorganisms in the compost may also provide nutrients for the larvae, allowing completion of larval stages and pupation into adults. Sciarid fly emergence is lower on unspawned sterile compost (lacking microbial activity) than on unspawned pasteurized compost (Kielbasa and Snetsinger 1981). When both organisms (*A. bisporus* and *L. ingenua*) develop on mushroom compost, competition for microbial nutrient sources may occur. A fully spawned compost with *A. bisporus* may be lacking the necessary nutrients for larval development.

When fully spawned compost was inoculated with *T. aggressivum*, sciarid adult emergence was higher in comparison to the fully spawned compost alone. Larvae also performed better (faster development) in comparison to the two other substrates. *Trichoderma aggressivum* may have rendered the fully spawned compost more suitable for larval nutrient uptake through secretion of degrading enzymes such as chitinases and cellulases that are responsible for its saprophytic activity (Guthrie and Castle 2006; O'Brien et al. 2014). Areas of the mushroom compost infested with green mold have a soft texture, often close to a liquid consistency. Saprophytic insects such as *L. ingenua*

feed from microbe rich host substrates and in fact benefit from microbe rich diets (Savopoulou-Soultani and Tzanakakis 1988; Mondy and Corio-Costet 2000; Becher et al. 2012; Witzgall et al. 2012; Yamada et al. 2015; Hamby and Becher 2016). The benefits that microbes render are related nutrition in the sense that microbes are consumed along with the food substrate and/or in the form of substrate pre-digestion, as a result of microbe degradation processes (Mondy and Corio-Costet 2000; Rohlfs and Kürschner 2010). Preliminary studies done in our lab found that larval development to the adult stage is also faster on unspawned compost inoculated with *T. aggressivum* compared to unspawned compost alone. This supports the notion that *T. aggressivum* provides additional nutrition value to *L. ingenua* in an already nutrient rich substrate like unspawned compost, hence, the larvae may benefit from increased microbe abundance.

Male development time was shorter in the presence of *T. aggressivum* green mold, compared to the other two substrates lacking *T. aggressivum*. The majority of the benefit in developmental time for sciarid fly populations is driven by the males (male developmental time is faster on *T. aggressivum*). Male sciarids are protandrous, regardless of the substrate they develop on. Once emerged, the males hover over the compost until the female flies begin to emerge (within approximately a 24 hour difference). As soon as the female fly emerges from the compost mating takes place. Males that emerge from green mold infested compost patches will likely mate sooner than those emerging at a later time. A faster developmental time driven by the presence of *T. aggressivum* may improve reproductive success, resulting in both males and females that are available for mating sooner. Further studies might determine the impact of a *T. aggressivum* diet on the F1 generation of sciarid flies.

Females that emerged from the spawned compost inoculated with *T. aggressivum* more were of a larger body size. O'Conner and Keil (2005) found a linear relationship between the weight of *L. ingenua* females and fecundity, as well as an effect of diet on female size.

The antagonistic and mycoparasitic mechanisms of *T. aggressivum* towards *A. bisporus* are becoming well known (Anderson et al. 2001; Savoie et al. 2001; Guthrie and Castle 2006; Krupke et al. 2003; Largeteau and Savoie 2010). *T. aggressivum* produces chymo-elastase and trypsin- like proteases during its interaction with *A. bisporus* all of which have been attributed to the pathogen's ability to attack *A. bisporus* (Williams et al., 2003*)*. Krupke et al (2003) suggests that *T. aggressivum* produces antifungal compounds which inhibit the growth and fruiting of *A. bisporus*, without causing death, rather, *T. aggressivum* utilizes nutrients liberated from the compost by *A. bisporus* extracellular enzymes, which suggests both a parasitic and an antagonist activity.

We have shown that sciarid larvae developing in compost colonized with *A. bisporus* and parasitized by *T. aggressivum* had increased larval fitness over those developing in compost with *A. bisporus* alone, indicating that sciarids may be benefiting from this parasitic and or antagonistic activity. One mechanism could be that T. *aggressivum* is suppressing the defenses of *Agaricus* mycelium. Larvae of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) like *L. ingenua,* benefit from the nutritional effects of a microbe rich diet (Rohlfs and Kürschner 2010). These larvae also benefit from the ability of microbes to impair the fitness of certain filamentous fungi that compete with the fruit flies (Rohlfs 2005). Something similar may happen when mushroom sciarid larvae consume a green mold infested spawned compost, where the *A. bisporus* mycelia which competes with *L. ingenua* is impaired by *T. aggressivum*. The mechanisms behind the negative effect of a fully grown *A. bisporus* mycelium on *L. ingenua* is not yet known. However, O'Conner and Keil (2005) found variability in *L. ingenua* development and survival within different mushroom strains, suggesting not only a nutritional effect, but one attributed to the nature of defense mechanisms among the mushroom strains tested. *Agaricus bisporus* chemical defense mechanisms against mushroom flies is an area yet to be explored. Past studies have suggested that *A. bisporus* antagonism towards sciarid flies may be due to the production of calcium oxalate

crystals, which coat developing hyphae in later stages of development and may create a mechanical barrier (Binns 1980). Fungal chemical defenses strategies against fungivores include hydrolysis of esters, oxidation of phenols and lipid peroxidation (Spiteller 2008). In Basidiomycetes such as *Agaricus*, the upregulation of proteins such as polyphenol oxidases is a response to physical wounding and attack by pathogens (Mayer 2006; Meng et al. 2012). More work is required to examine *A. bisporus* chemical defense mechanisms against fungivores and how the production of defense related compounds is affected by *T. aggressivum* antagonism and parasitism.

Conclusions

The association between *L. ingenua* and *T. aggressivum* commonly found in mushroom farms is of a mutualistic nature. This emphasizes the importance of *L. ingenua* management in commercial mushroom farming. Likewise, the control of mushroom green mold plays a role in sciarid fly management, given that it is a microbe that promotes *L.ingenua* development. Revealing the underlying mechanisms of this insect-microbe association can deepen our understanding of the roles that microbes play in insect ecology. The latter may be useful in suggesting the potential applications of microbes, such as *T. aggressivum* in sciarid fly management, for example, the development of a microbe-based attractant for targeted pest control or pest population monitoring.

Chapter 3

Mushroom Sciarid Fly, Lycoriella ingenua (Diptera: Sciaridae) adults and larvae vector Mushroom Green Mold (Trichoderma aggressivum ft. aggressivum) spores.

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Abstract

Gravid female mushroom sciarid flies (*Lycoriella ingenua*, Dufour, Diptera: Sciaridae) were confirmed in laboratory experiments as vectors for the fungal pathogen *T. aggressivum* ft. *aggressivum* Samuels & Gams, causal agent of mushroom green mold disease in mushroom farming. The gravid females acquired the fungal spores when exposed to *T. aggressivum* cultures, which was confirmed by scanning electron microscopy, and flies vectored *T. aggressivum* onto clean agar petri dishes, confirmed by new fungal colonies growing on the media and molecular analysis (PCR). Significantly more *T. aggressivum* colonies formed on the dishes when flies were left to vector the pathogen for 30 hrs, as opposed to 18 hrs, indicating that females' 24 hour pre-oviposition period limited fly movement. One gravid female fly was able to initiate up to 32 *T. aggressivum* colonies in a 0.3 m^2 area. Frass deposits of mushroom sciarid fly larva reared on *T. aggressivum* contained viable spores, detected through fungal subcultures and molecular analysis (PCR), confirming that larvae can also vector the fungus. This study

supports the heretofore anecdotal evidence that mushroom sciarid flies are part of green mold disease epidemiology on mushroom farms.

Introduction

A major worldwide threat to mushroom farming is the potentially devastating green mold disease, caused by the fungal pathogen *Trichoderma aggressivum* ft *aggressivum* Samuels & Gams (North America) and *T. aggressivum* ft *europaeum* Samuels & Gams (Europe). Green mold first appeared in Pennsylvania, USA, in the early 1990's in cultivated mushroom beds (Anderson et al., 2001). The most recent Green mold losses in the United States were estimated to be $14 million USD in 2011 (Pecchia, 2012).

Green mold disease is characterized by a dense grey hyphal growth on mushroom compost or casing materials that later turns white followed by sporulation which gives a dark green color to the infection. There can be a complete lack of mushroom production in infected areas (Anderson et al., 2001; Coles & Barber, 2002; Guthrie & Castle, 2006). Many factors have been associated with the occurrence of green mold disease in mushroom farming including the nature of the raw materials used for the mushroom growing substrate and composting procedures, reduced attention to sanitation and poor post-crop steam off programs (Anderson et al., 2001). However, no specific compost or environmental conditions have been found to be consistently associated with green mold development, and the mode of infection routes are poorly understood. Spores are easily carried by contaminated equipment and substrate materials that have not been properly pasteurized,

or contaminated materials from harvesting such as mushroom trimmings. Spores can also be carried by farmworkers via clothing (Seaby, 1996; Coles & Barber 2002) .

Mushroom flies have only been anecdotally implicated in the spread of green mold disease. *Trichoderma aggressivum* spores are contained in a 'sticky matrix' that can easily attach to the fly bodies (Anderson et al., 2001; Coles & Barber 2002). The flies may acquire the spores when they land on compost patches infested with *T. aggressivum* then spread the spores to other areas of the compost bed through further movement. Growers in Kennet Square, PA, have noted an association between fly populations and green mold disease incidence inside mushroom farming houses (personal communication). Female *L. ingenua* have been shown to be ovipositionally attracted to *T. aggressivum* on mushroom compost (Cloonan et al.,2016) opposed to mushroom compost alone. In addition, Mazin et al (2018) showed that mushroom sciarid larvae reared on spawned compost infested with green mold are more successful at reaching adulthood and develop faster into adults than those reared on spawned compost alone.

The mushroom sciarid fly *Lycoriella ingenua* is a significant pest in its own right in mushroom farming worldwide. Female *L. ingenua* flies enter the mushroom growing rooms soon after they are filled with pasteurized (unspawned or spawned) compost and continue to infest the growing rooms until the first days of spawn run (stage of active *Agaricus bisporus* mycelial growth in the compost) (Coles, 2002). Once inside the growing rooms, the flies readily lay eggs on the compost. Larvae hatch ca. 21 days later and begin to feed on the compost.

The severity of green mold disease is related to the timing of *T. aggressivum* infection during the crop cycle. Higher yield losses occur when green mold sporulation is

detected early in the crop cycle (during spawn run and casing stages) (Anderson et al., 2001). A potential synchronicity exists between the time of *T. aggressivum* infestation and the invasion of *L. ingenua* females during the first days spawn run. Given that *L. ingenua* females are attracted to green mold, there is a risk of disease spread through the fly's mechanical vectoring of spores (through movement) at the most susceptible stage of the crop.

External acquisition (attached to the exoskeleton) and dispersal (vectoring) of fungal spores onto crops by dipterans has been studied in shore flies (Ephrididae), moth flies (Psychodidae) and fungus gnats (Sciaridae) (El-Hamalawi & Stanghellini, 2005; El-Hamalawi, 2008; Scarlett et al.,2014). Some studies have also described larval acquisition of fungal propagules by ingestion and excretion of viable spores through frass deposits (Gardiner et al., 1990; Jarvis et al.,, 1993; Stanghellini & Rasmussen, 1996; Braun et al., 2012).

Shamshad et al (2009) documented the vectoring of another recurring disease in mushroom farming (dry bubble disease caused by *Lecanicillium fungicola*) by *L. ingenua*. Seaby (1996) found that *T. aggressivum* biotype T2 grew from the bodies of *L. ingenua* adults collected from mushroom houses in Europe. No information exists regarding the role of *L. ingenua* adults as mechanical vectors, nor has the role of larvae as vectors of *T. aggressivum* been explored.

The objectives of this study were: 1) the characterization of the external acquisition of *T. aggressivum* spores by *L. ingenua* when exposed to *T. aggressivum* ft *aggressivum* 2) the extent and efficiency of *T. aggressivum* spore dispersal by gravid *L. ingenua*, especially the formation of *T. aggressivum* colonies in space and time due to fly vectoring of spores.

3) To determine whether *L. ingenua* larvae are also vectors of green mold spores through their consumption and excretion of *T. aggressivum*.

Materials and methods

Sciarid flies

The flies used for this experiment were from a 4-year-old laboratory colony maintained at the Department of Entomology, University Park Campus of Pennsylvania State University. The flies were reared on a mixture of phase II mushroom compost (prepared at the Mushroom Research Center, Pennsylvania State University) and an addition of a nitrogen supplement (100:1, w/w) in an environmental growth chamber at 21 °C, 70% r.h., and L12:D12 photoperiod.

Trichoderma aggressivum cultures

The *T. aggressivum* ft. *aggressivum* cultures used for this study were derived from a stock culture (#324#; Chester County Farm; Romaine) kept in the disease collection of the Department of Plant Pathology and Environmental Microbiology (Pennsylvania State University). Subcultures were grown in 9 ml plastic petri dishes containing malt extract agar, antibiotics (Kanamycin Sulfate 100 μg/ml, Thermo Scientific) and Tween, and incubated in total darkness at 25°C. Once *T. aggressivum* colonies began to sporulate (approximately 3 days later) the dishes were used in the experiment.

Distribution of T. aggressivum spores on adult flies with scanning electron microscopy

Twenty *L. ingenua* females were collected using manual aspirators from a laboratory colony (described above). From this group of flies, ten were randomly selected as treatment subjects and placed individually into petri dishes containing cultures of *T. aggressivum* ft *aggressivum* (described above). The other ten flies served as controls and were introduced individually into petri dishes containing only water agar. All flies were kept in the petri dishes for 20 minutes, after which, they were collected with an aspirator, placed individually in sterile test tubes and freeze killed at -20°C for 1 hour.

Individual flies were mounted on scanning electron microscopy (SEM) stubs with no coating to prevent obscuring spores. The flies were examined using a FEI Nova NanoSEM 630 FESEM scanning microscope, with a magnification of 7X, from the materials characterization lab at the Penn State University.

Adult spore vectoring

Individual sciarid fly pupae were collected manually from the laboratory colony and placed into sterile glass test tubes which were kept in an environmental growth chamber at 21°C, 70% r.h., and L12:D12 photoperiod and checked daily for adult emergence. Upon emergence 4-5 days later, adult flies were sexed based on morphological characters (Lewandowsky et al. 2004). Fly pairs were placed into test tubes for mating. Once mating occurred, the pairs were separated and the gravid females were kept isolated in individual test tubes for use in the experiment.

Females reportedly have a 24-hour pre-oviposition period (McDonald, 1972). For this reason we established two time treatments to correspond with ovipositon status, one in which female sciarid flies were kept in the experimental chambers for 18 hours (pre-oviposition treatment) and another treatment in which flies were left in the experimental chambers for 30 hours (post-oviposition treatment). Twenty gravid female *L. ingenua* flies were used in each treatment.

For each replicate, one gravid fly was released into an experimental chamber (10 x 30 cm tightly sealed plastic box). The chambers contained a 9 ml petri dish with a fully sporulated *T. aggressivum* culture (described above) and six 9 ml plastic open petri dishes containing malt extract agar with antibiotics, arranged in three rows of 2 petri dishes. Each row was 10, 20 and 30 cm from the inoculum source (petri dish with sporulating *T. aggressivum*), respectively.

We established two controls: 1) an experimental chamber containing six open petri dishes with malt extract agar and antibiotics and one gravid fly but no *T. aggressivum* dish and 2) an experimental chamber containing six open petri dishes with malt extract agar and antibiotics, an open *T. aggressivum* inoculum dish but no gravid fly.

After releasing the flies, the experimental chambers were placed in a completely randomized design in an environmental growth chamber at 21°C, 70% r.h., and L12:D12 photoperiod. After 18 or 30 hours the petri dishes were removed from the chambers, covered with lids and placed in an incubator at 25°C. The dishes were inspected daily until *T. aggressivum* colonies became visible (after approximately 2-3 days of incubation). The number of *T. aggressivum* colonies on each plate was counted and recorded for a total of 5 days.

Due to growth chamber space constraints, the experiment was conducted in rounds. In each round, two 18 hour treatment chambers and two 30 hour treatment chambers were tested along with both controls (1 of each per treatment). In total, 20 gravid female flies per treatment were tested divided in ten different rounds.

Larval vectoring experiment

Ten one-day-old adult female sciarid flies from the laboratory colony were collected and allowed to oviposit onto water agar in petri dishes. Upon eclosion (ca. 5 days after oviposition) twenty neonates were placed individually into portion cups (4 cm diameter, 3 cm high; Dart Solo, Harrisburg, PA, USA) containing a malt extract agar cutlet from a sporulating *T. aggressivum* culture (c.a. 5 days old). Cups with larvae were kept in an environmental growth chamber at 21°C, 70% r.h., and a L12:D12 photoperiod and allowed to feed on the *T. aggressivum* mycelia until they reached the third instar. The larvae were only left to the third instar because it was observed that *L. ingenua* larvae typically cannot reach pupation when left feeding on fungal mycelia grown on agar alone (unpublished). In addition, first and second instar larvae are too small to withstand surface sterilization. Once the third instar was reached, each larva was removed from the portion cup and, modifying the method of El- Hamalawi (2009), surface sterilized by drenching them with 2-3 drops of 0.5% sodium hypochlorite followed by a rinse in distilled water.

Immediately after surface sterilization larvae were transferred onto petri dishes containing water agar for excretion. After 24 hours, larval frass was observed. A sample of twelve larval frass deposits was collected and plated individually onto petri dishes

containing malt extract agar. The dishes were then placed in an incubator at 25°C. *Trichoderma aggressivum* spores present in the frass deposits were considered viable if they germinated and grew after 5 days of incubation at 25°C (Samuels et al., 2002).

The efficacy of the surface sterilization technique described above was tested prior to conducting the experiment. Ten sciarid larvae were surface sterilized, freeze killed and placed individually on 9 ml plastic petri dishes containing malt extract agar. The dishes were incubated at 25°C for 5 days. Since no fungal colonies grew on the dishes, the sterilization was considered effective.

Molecular Pathogen Confirmation

Confirmation of fungal species from plated frass colonies was determined by PCR. Fifteen plates from the larval experiment and 10 plates with mycelial growth from the adult vectoring experiment were randomly selected for molecular characterization. Subcultures (consisting of agar cubes) were taken from these samples and plated on glucose yeast extract tyrosine agar (GYET) as well as onto potato dextrose broth (PDB). Subcultures were deemed ready for extraction when fungal growth completely covered the surface of the broth. The fungal tissue was aseptically retrieved from the tube and placed into a sterile 1.5 mL centrifuge tube. A Genomic Wizard DNA Extraction kit was used (Promega, Madison, WI) to perform a total DNA extraction of the fungal material. Due to the specific nature of the PCR protocol being used, *T. aggressivum* did not need to be in pure culture. PCR was performed on a Nyxtechnik (San Diego, CA) thermocycler using a protocol and primers previously developed at Penn State to detect *T. aggressivum* in samples (Chen et

al. 1999). Samples were heated at 94°C for 2 minutes, followed by 35 cycles of 94°C, and 60°C at 1 minute each. A final stage of 70°C for 7 minutes occurred, before samples were held at 4°C. Two sets of primers were used. Primers GenTrich-F (5'-GTTGGTTCTGCCTTCTGG-3') and GenTrich-R (5'-AACAGCTGGCCAAAGGGG-3') for the detection of any species of *Trichoderma*, and Th2/4-F (5'-CGGTGACATCTGAAAAGTCGTC-3') and Th2/4-R (5'-TGTCACCCGTTCGGATCATCCG-3') for the detection of *T. aggressivum.* The size of the fragments differentiates the amplicon for both sets of primers, with the general *Trichoderma* amplicon being 369 bp, and the *T. aggressivum* amplicon being 444 bp. After PCR amplification, the samples were run on a 2% agarose gel until sufficient band separation was observed. Gels were imaged on a gel imager (Alpha Innotech MultiImage II), and photographs were saved for further evaluation. To confirm the band results, two positive controls were run in each row of the gel. If the sample bands matched those of the control, the sample was rated positive for *T. aggressivum*.

Statistical analysis

The effect of vectoring time (based on oviposition status) and distance from the inoculum source on the number on *T. aggressivum* colonies was analysed with a main effects linear model (GLM, SPSS v 24) with an interaction between vectoring time and distance from inoculum source.

Results

Detection of T. aggressivum spores on adult flies with scanning electron microscopy

SEM images consistently revealed fungal spores on the femorotibial joint setae and tarsi of all flies introduced onto *T. aggressivum* culture plates, with a low number of spores observed on the ovipositors. No spores were found attached to the exoskeleton of the control flies (Fig. 4)

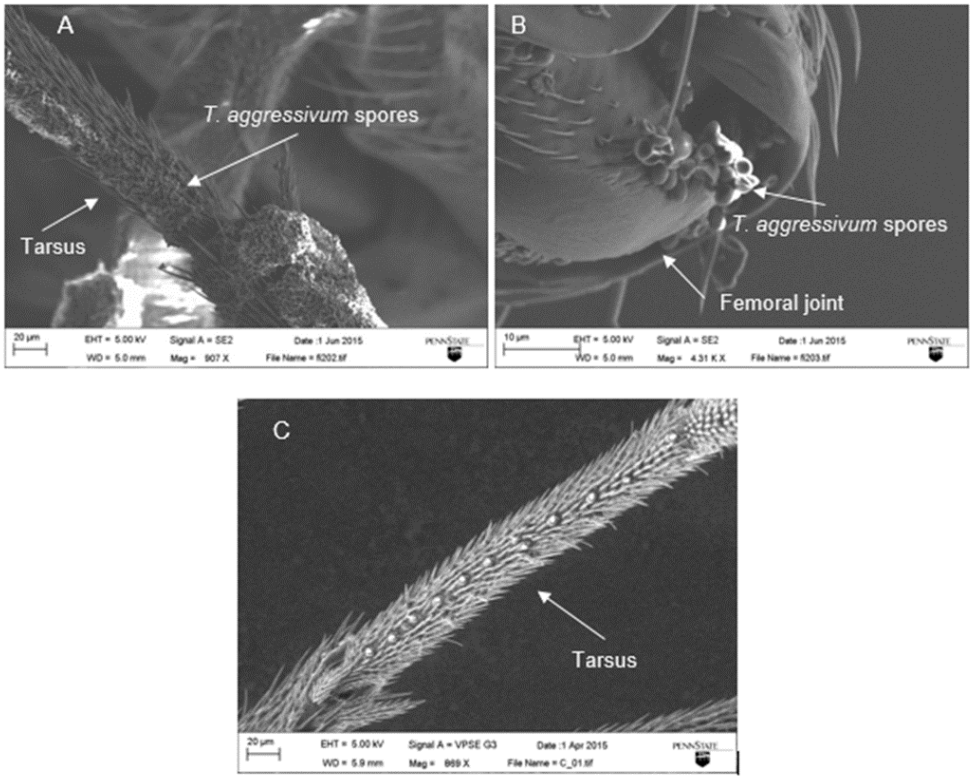

Figure 4: SEM images of fungal spores on the tarsi (A) and femur joint (B) of an *L. ingenua* female after being exposed to a sporulating culture of *T. aggressivum*, no spores were detected on control flies (C)

Adult vectoring study

No *T. aggressivum* colonies developed on the petri dishes within the control treatments negating the airborne transmission route.

The general linear model analysis (table 1) shows that the number of colonies between time intervals differed significantly. Gravid female sciarid flies vectored more *T. aggressivum* when left in the experimental chamber for 30 hours (3.4 ± 0.23 S.E.) than when left to vector for 18 hours (0.54 ± 0.007 S.E.) $p < 0.05$) (Fig. 6). The petri dishes that were in the experimental chambers where both the pathogen and the flies were present began yielding fungal growth 24 hours after being placed in the incubator. After 2 days, *T. aggressivum* colonies were fully formed and visible (Fig. 5).

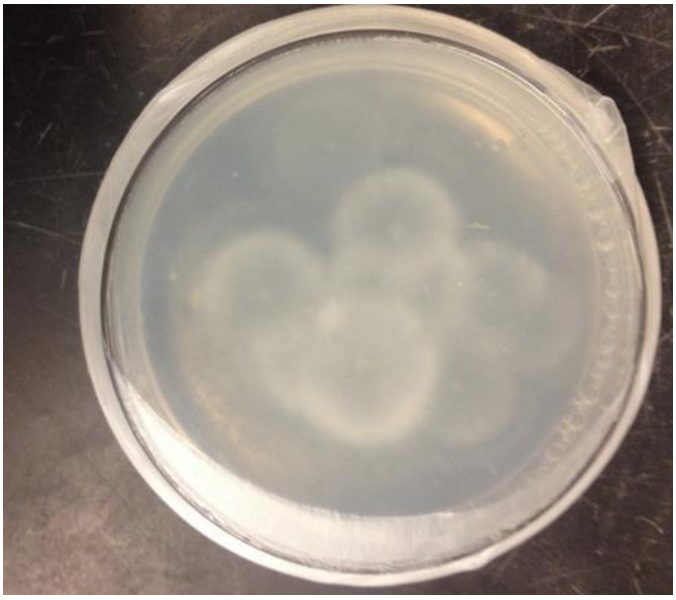

Figure 5**:** Fungal colony formations on a petri dish after being exposed to a gravid female fly infested with *T. aggressivum* for 30 hours, image shows various colonies originated through fly movement

There was no significant effect of distance (up to 30 cm) from the inoculum source on the mean number of *T. aggressivum* colonies vectored by *L. ingenua*. Likewise, there was no interaction between the time of exposure and the distance from the inoculum source (Table 1).

Molecular analysis of the colonies from these dishes confirmed positive for *T. aggressivum*.

Source	Type III Sum of Squares	DF	Mean Square	F	Sig.
Corrected Model	523.071[a]	5	104.614	29.585	0.000
Time	501.704	1	501.704	141.884	0.000
Distance	10.758	2	5.379	1.521	0.221
Time * Distance	10.608	2	5.304	1.5	0.225
Error	827.425	234	3.536		
Total	2267	240			
Corrected Total	1350.5	239			

a. R Squared = .387 (Adjusted R Squared = .374)

Table 1: Between subjects effects (GLM) of number of *T. aggressivum* colonies by vectoring time and distance from inoculum source

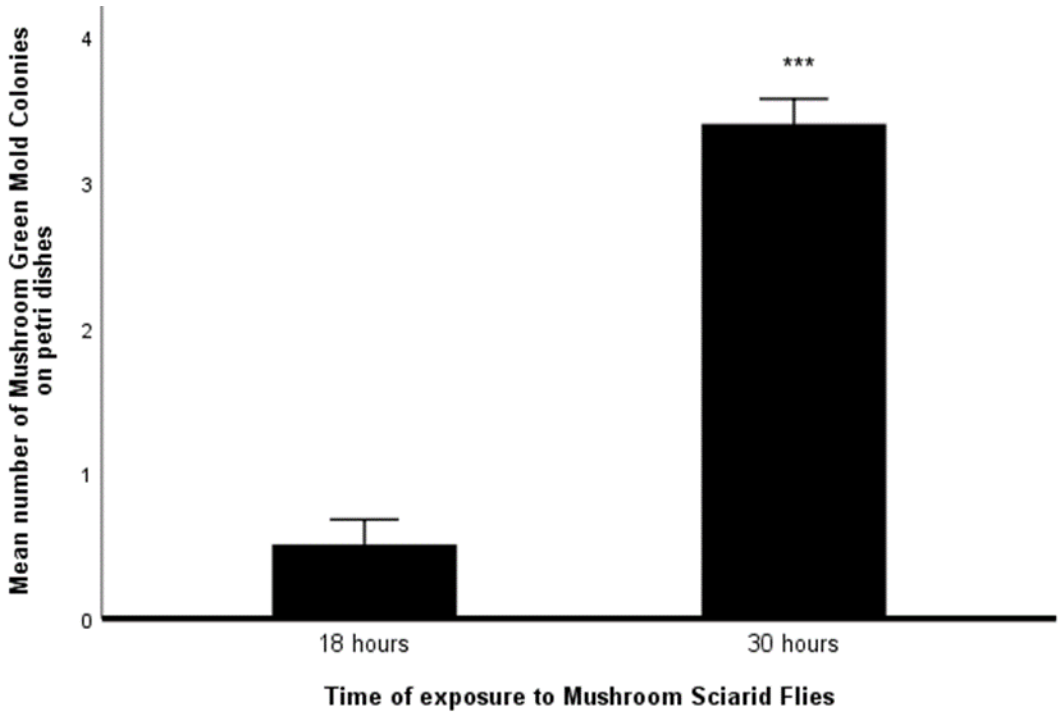

Figure 6: Mean number (± S.E.) of *T. aggressivum* colonies on petri dishes exposed to green mold infested *L. ingenua* gravid females for 18 and 30 hours. Bars with different lettering denote significant differences among means (GLM < 0.05).SEM images of fungal spores on the tarsi (A) and femur joint (B) of an *L. ingenua* female after being exposed to a sporulating culture of *T. aggressivum*, no spores were detected on control flies (C).

Larval consumption and excretion of *T. aggressivum* spores

All larvae were able to feed on *T. aggressivum* mycelia until the third instar (after which larvae were removed and surface sterilized). Frass deposits were collected from all twenty larvae (Fig. 7). All of the plates containing frass deposits showed mycelial growth

after c.a 2-3 days. Molecular analysis of the subcultures from these dishes confirmed positive for *T. aggressivum*.

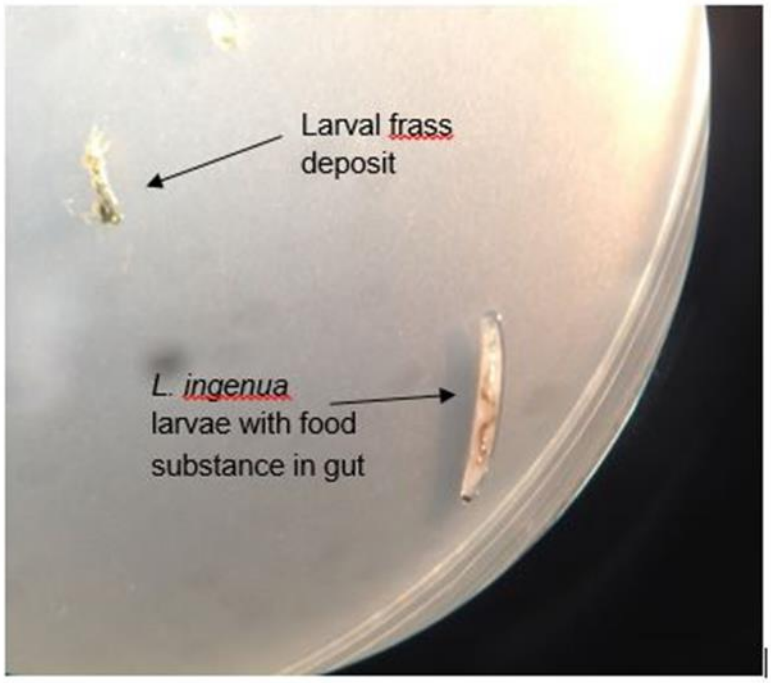

Figure 7: Larva of *L. ingenua* on a petri dish after having fed on *T.aggressivum* mycelia, surface sterilized and allowed to deposit frass. The intestinal tract of the larvae is dark with food substance. All larvae produced frass deposits which contained viable *T. aggressivum* spores.

Molecular Pathogen Confirmation

PCR results confirmed that viable propagules of *T. aggressivum* were found in all samples from both larval frass and adult flies. The band density in the gel was much lower in the adult samples compared to the frass samples, possibly indicating less *T. aggressivum*

material present on the adults. However, as the study was not set up for quantitative comparison, this cannot be confirmed.

Discussion

Our objectives were to document the acquisition and spread of *T. aggressivum* spores by the female scarid fly *L .ingenua* onto a new surface (in this case, growing media). The SEM data provided visual confirmation of the acquisition of the spores by the female fly after being exposed to sporulating *T. aggressivum* cultures. Although this study did not quantify the number of spores on the flies, there appeared to be more spores on the tarsi and joints of the flies than on other parts of the body, such as the ovipositor. Perhaps spore acquisition is a sole result of the female landing and walking on the *T. aggressivum* colonies rather than an intentional gathering of the spores. However, we have observed a grooming - type behaviour when a sciarid female comes into contact with *T. aggressivum* spores, possibly indicating active spore harvesting by the fly. This is yet to be confirmed.

The growth of fungal colonies on the petri dishes that were exposed to both the pathogen and the flies indicate role of the fly in vectoring of *T. aggressivum* by *L. ingenua*. This is further supported by the control experiments where flies were not present, which yielded no fungal colonies, eliminating the airborne transmission. Female sciarid flies are attracted to mushroom compost infested with *T. aggressivum* as opposed to un-infested mushroom compost (Cloonan et al. 2016). Our data confirm that the flies visited the petri plates inoculated with *T. aggressivum* once released in the chamber and that the spores

adhered to their exoskeleton upon landing at least passively, if not actively harvested, after which they visisted to the clean petri dishes causing green mold infections

The marked difference between the number of colonies formed when the flies were left to vector for 18 versus 36 hours demonstrates that time affects the amount of pathogen dispersal. These results support the work by Hamalawi et al (2008) who found that the total number of colonies of *T. basicola* developing on petri plates increased with increased exposure time to the infested sciarid fly, *Bradysia impatiens* (Johannsen) (Diptera: Sciaridae). During the experiment, we often observed that *L. ingenua* females will remain almost entirely immobile for approximately 24 hours after mating (this was visually determined by inspecting the fly's location in the chamber every couple hours, however, these observations were not carried out systematically for all chambers). The low number of colonies that resulted from 18 hours of exposure time may be due to a lack of movement during this pre-oviposition period.

The poor correlation between the number of fungal colonies on a petri dish and the distance from the inoculum source is perhaps a result of size of the experimental chambers, causing the flies to saturate the space provided. Perhaps if the chambers were larger in longitude, we may have seen a difference in a linear one.

Our results demonstrated that *T. aggressivum* can also be vectored through larval frass deposits. After eclosion from the egg, sciarid fly larvae typically migrate downwards through the compost, (sometimes going beyond 15 cm) and as they reach their final instars, they then migrate upwards (to the first 5 cm). Few larvae move further up into the casing layer (peat moss applied to the surface of the compost) once it is applied (Cantelo, 1988). This study focused on frass deposits produced by third instar larvae. We do not know

whether first and second instars produce green mold infested frass. Frass deposits from the larvae collected remained viable and were able to form new colonies in growing media, however this may not be the case in production conditions. It is not known how far larvae migrate horizontally though the mushroom compost, however, assuming that the spores remain viable in compost conditions, larval frass will possibly aggravate the development of the disease on a localized, longitudinal scale. Tuno (1998) documented spore dispersal of the ephemeral mushroom *Dyctiophora* sp. through the frass deposits of drosophilids (Diptera: Drosophilidae). Fungal spores were found inside the anus of the flies, and these spores were able to initiate new colonies, the author reported that larvae's digestive processes did not affect spore viability.

The few green mold epidemiology studies in mushroom farms have not included sciarid fly population levels. Royse et al (1999) found a non-random pattern of green mold disease foci (mostly in occurring in aggregated patterns in adjacent sections along the mushroom beds) and concluded that *T. aggressivum* inocula are spread by workers and contaminated equipment. Rinker et al (1996) reported that only a few *T. aggressivum* conidia are carried by the air emphasizing the spread of green mold disease through a carrier, rather than wind or dust particles as postulated by Seaby (1993). Our experiment supports these findings given that no colonies were formed on the petri dishes from chambers containing an inoculum source with no flies. Our experiment confirms that *L. ingenua* adults and larvae are able to spread *T. aggressivum* spores from infested compost to agar. If this is the case in commercial mushroom farming conditions, it is likely that *L. ingenua* contributes to the occurrence and spread of green mold disease in mushroom farms. These findings emphasize the importance of fly control, in both the larval and adult

stages. Our results also imply that there may be a time period of approximately 18 hours in which the implementation of adult fly control measures are crucial, given that perhaps the spread of *T. aggressivum* due to fly movement will begin as early as 18 hours after initial female fly detection.

Conclusions

Certainly, many factors contribute to the development of green mold disease in a mushroom farm and no sole factor acts alone in green mold epidemiology. This study confirms suspicions that mushroom sciarid flies are vectors of *T. aggressivum* spores. Although further study is needed to determine the degree to which mushroom flies contribute to the development of mushroom green mold disease in commercial farming conditions, our findings emphasize the need for timely sciarid fly management as a control measure for *T. aggressivum*.

Chapter 4

Activity and Distribution of the Mushroom Phorid Fly, *Megaselia halterata*, In and Around Commercial Mushroom Farms

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Entomologia Experimentalis et Applicata, in press

Abstract

The mushroom phorid fly, *Megaselia halterata* (Diptera: Phoridae), is a key pest in mushroom farming in most parts of the world. Studies on the mushroom phorid fly have focused on its life history within mushroom growing houses, but little is known about the fly's activity outside mushroom growing houses. We studied activity and distribution of adult *M. halterata* in the areas surrounding mushroom growing houses using yellow sticky traps. *M. halterata* focuses its flight activity over turf areas rather than windbreaks and spent compost piles, possibly for mating purposes. No evidence was found that *M. halterata* was ovipositing in turf areas surrounding mushroom growing houses. In addition, flight activity was highest in the afternoon until midnight at higher temperatures yet at lower temperatures activity ceased after sunset. Establishing temperature and daylight thresholds for *M. halterata* flight activity may be useful in developing IPM tactics for this species. The most successful IPM tool that mushroom growers use at present is fly exclusion. Exclusion can be improved by focusing farm operations around temperature and daylight thresholds when fly activity is at its lowest.

Introduction

The mushroom phorid fly, *Megaselia halterata* (Wood) (Diptera: Phoridae) is a key pest in mushroom farming in most parts of the world (Richardson & Hesling, 1978; Keil 2002). Studies on the mushroom phorid fly have focused on its life history within mushroom growing houses , but little is known about the activity of *M. halterata* outside mushroom growing houses, including nearby residential neighborhoods where the fly can become a serious nuisance to homeowners (Binns et al., 1979). Mushroom growing is performed year round inside growing rooms, where *M. halterata* populations also fluctuate year round. Mushroom growers in Chester County Pennsylvania (USA), where our study was performed, report that *M. halterata* populations begin to build up during the late summer months (June and July) and become a problem during fall (from August to November), when they reach their highest levels. The populations then begin to decrease in December and remain low during the winter and spring months. Also, little is known about the activities and behavior of feral populations of this pest species. *Megaselia halterata* are obligate fungal feeders (Scheepmaker 1996), and hence females are attracted to spawned mushroom compost (compost with active mycelial growth) (Tibbles et al., 2005). Some reports suggest that female phorids enter rooms at "spawn run" (stage in the mushroom production process after which the compost has been 'seeded' and the mushroom mycelia are actively growing in the compost) either from outside the mushroom-growing house or from other growing rooms within the house (Hussey, 1960; Binns 1979; Navarro et al., 2001). Once inside the mushroom house,

females lay eggs on the mushroom mycelia growing in the spawned compost and neonates feed on the mycelia (Keil 2002).

Activity patterns of *M. halterata* flies have been studied on commercial mushroom farms. Hussey (1965) trapped mushroom phorid flies outside mushroom growing houses with suction traps and concluded that phorid fly flight is governed by both temperature and daylight. He found that the critical threshold for flight outside growing houses is an air temperature of 12.8° C, however, flight does not become 'general' until the air reaches a temperature of 15.5° C. In morning hours after sunrise, phorid flies will not become active until air temperatures go above 12.8° C. In the evenings, flight will be curtailed by sunset, even when the temperature may be above the threshold for flight (Hussey, 1965). Jess et al (2007) studied *M. halterata* activity on mushroom compost wharfs (exterior open spaces where mushroom compost is processed) and found little to no activity of the flies in Phase I and Phase II composting areas, or at the bagging area where compost is placed into bags before being transported to the farms. They concluded that phase II mushroom compost is not a source of *M. halterata* for infestations within growing houses.

Anecdotal reports of *M. halterata* describe large numbers in what appear to be swarms outside of mushroom houses (Hussey, 1965). Our preliminary observations on mushroom farms in Chester County, PA, place this "swarming" behavior over mowed turf areas on the farms. Swarms may indicate mating behavior as documented in other fly species such as chironomids (Fyodorova & Azovsky, 2003) or mosquitoes (Hassan et al.,2013; Tuten et al., 2013) or alternatively, these are just large populations of *M. halterata* leaving the mushroom houses and flying around before feeding, laying eggs, or

dying. Hussey & Gurney (1964) reported that a period of flight is required for *M. halterata* before mating, although this reporting is from laboratory rearing studies. The mating status of flies within these large populations outside the houses has not been reported.

In addition to turf areas, other typical landscapes around mushroom houses may be associated with phorids. These include wooded areas at the perimeters of the farm properties as well as collections of used ("spent") compost outside the houses. Some farms keep this spent compost, a waste product of production, on the farm premises for several days or more until it is transported off the farm. Spent compost has been anecdotally suspected of being a source of *M. halterata* infestations of subsequent mushroom crops.

The objectives of this study were to gain a greater understanding of the daily activity and distribution of adult *M. halterata* in the areas surrounding mushroom growing houses by determining where and when flies are found in the greatest numbers. Specifically, our objectives were 1) to compare *M.halterata* adult activity among turf areas, windbreak areas and spent mushroom compost piles, all surrounding commercial mushroom growing farms 2) to determine diurnal fly activity around the farms 3) to determine whether mushroom phorid fly larvae could be found in the turf areas surrounding the farms. Ultimately, results from the study may inform the development of new tactics to control infestations and reduce annoyances to surrounding residential neighborhoods.

Materials and methods

We used yellow sticky traps (Alpha Scents Inc. 1089 Willamette Falls Drive West Linn, OR 97068, USA) to determine the relative distribution of *M. halterata* adults in the following areas that typically surround mushroom growing houses: 1) grassy turf areas near the mushroom houses; 2) near wind break areas farther away from the houses; and 3) over piles of spent mushroom compost immediately outside the houses. We also used yellow sticky traps to determine the daily *M. halterata* adult activity patterns outside the mushroom houses during 'early season', in the month of August and during colder periods later in the season in the month of October. Finally, we observed the behavior of *M. halterata* adults during flights of large populations over turf areas to determine whether these flights were related to mating behavior or not.

Four mushroom farms were used in this study. All farms were located in Chester County, PA, and all were within 7 to 27 kilometers of each other. Trapping experiments were conducted during two different times of the year, a warm period from August 22 to August 26, 2016, and a colder period from October 5 to October 7, 2016. Each sticky trap used was a double-sided yellow panel, 18 X 14 cm with dry adhesive coating both sides of the panel. The traps were deployed using short metal stakes pushed into the ground. The bottoms of the sticky cards were between 10 and 15 cm off the ground.

Trapping at differing sites and distances from mushroom houses

All four of the mushroom houses used for this study had areas of turf (mowed lawn-type grass) located 15 m and 30 m (3 farms) and 90 m (1 farm) from the walls of

the house. However, only two of the four mushroom houses had turf extending out to narrow windbreaks (10 meters wide) containing several species of mature deciduous and evergreen trees as well as spent compost piles on the farm premises, so only these two farms were used for the trap-site comparison study in which we compared captures from compost piles, turf-near-mushroom-houses, and turf-near-windbreak areas. Thus, for this trap-site-difference test performed in August, on Farms 1 and 2, two yellow sticky cards were deployed on spent compost piles, on turf near the mushroom houses, and on turf areas at the edge of windbreaks, farther away from the houses. On Farm 1 the distances from the house were: 1) spent compost, 30 m from the house; 2) turf 15 m from the house; 3) turf 90 m from the house at the edge of the windbreak. On Farm 2, the distances from the house were: 1) spent compost, 70 m from the house; 2) turf 15 m from the house; and 3) turf, 30 m from the house at the edge of the windbreak.

After deployment, each trap was photographed three times per day, with a high resolution digital camera to evaluate the outdoor time of flight activity of *M. halterata*. Traps were deployed at 4 PM on August 22 and photographed at 12 AM and 8 AM the next day. When traps were re-visited at 4 PM, the old traps were replaced with new traps. This procedure was repeated each day for the next five days. For the trap site comparison, phorid flies on each card were counted from the photos and the number captured per day on each of the two traps at each of these three sites on Farms 1 and 2 was recorded.

Daily flight activity

In August, trap captures over the turf areas nearest the mushroom houses on all four farms were used for assessment of daily flight activity. The traps deployed on Farms 1 and 2 were as described above, and on Farms 3 and 4, two traps each were placed in three different locations over turf areas on each farm. These traps were deployed 15 m and 52 m from Farm 3, and 15m, 16m, and 28 m from Farm 4. The three capture periods assessed each day thus were from 4 PM to 12 midnight, 12 midnight to 8 AM, and 8 AM to 4 PM.

In October, we concentrated on assessing daily fly activity using turf areas near the mushroom houses on the same turf areas used during August on each of the same four farms. This adjustment was made in response to the preponderance of *M. halterata* captures on the turf areas closest to mushroom houses during the sampling in August. Four daily trapping intervals (instead of three as in August) were used in October to more finely dissect any differences in daily flight activity. The intervals used during October were 6 AM to 10 AM; 10 AM to 4 PM; 4 PM to 8 PM; and 8 PM to 6 AM.

The October trapping test started on October 5 at 4 PM, with three yellow sticky traps being deployed on each of the four turf areas on the four farms. As in the August experiment, the sticky traps were placed at a height of 10 to 15 cm from the ground using metal stakes pushed into the turf. Traps were replaced with new, clean traps at each observation. Trapping stopped on October 7 at 10 AM. Collected traps were immediately covered in plastic film food wrap and returned to the laboratory at Penn State for counting.

For the daily flight activity studies, the number of *M. halterata* flies captured only on traps on the "turf near mushroom houses" on each of the four farms was used. For each time interval the number of *M. halterata* flies caught on each card was recorded.

Sampling of adults flying over turf for evidence of sexual activity.

On October 17th and 18th in 2016, between 5 and 7 pm, *H. halterata* adults were sweep-netted on or near turf on Farm # 2. The specimens that were netted were examined for pairs in copula. In addition, apparent in-flight pursuits by large numbers of flies following individual flies on the hood of our car parked next to the turf area were observed and video-recorded.

Turf samples for immatures

During the October sampling dates, 30 cubic cm of turf samples were taken from each the same turf areas where the traps were placed for monitoring phorid fly larval activity. Three samples, approximately 5 m apart, were taken using a post hole digger. Turf samples were placed into sterile polyethylene bags. In the lab, each sample bag was opened and placed into an emergence cage (cages (30 x 30 x 30 cm) with a single vinyl window (Raising Butterflies, UT, USA) housed in a growth chamber at 21 °C, 70% r.h., and L12:D12 photoperiod for 30 days. The cages were visually inspected for fly emergence on a daily basis. In addition, 3 random sub samples were taken from these turf

samples and dissected with forceps under a stereoscopic microscope for the presence of *M. halterata* immature stages.

Statistical analysis

For comparison of the number of mushroom phorid flies among different sites outside mushroom houses (data gathered in the month of August), we used a General Linear Model (p=0.05) and a Tukey's Multiple Comparison Test for mean separation. In order to compare time intervals tested for each month as well as the time intervals between months in August and October, the number of phorid flies per interval were converted to flies per hour. For each month, the effect of time interval on phorid fly activity was tested with a main effects general linear model with interactions. The variables tested were the time interval and the farm sampled as predictors of the number of phorid flies caught on the cards. Main effects were separated using a Tukey's Multiple Comparison Test. All statistical analyses were done using SPSS V.24. An alpha level of 0.05 was selected.

For reference, hourly temperature values from the nearest weather station in August 22-26, 2016 and October 5-7, 2016 were obtained from the National Oceanic and Atmospheric Administration (NOAA) (Databases: Local Climatological Data Hourly Observations August 2016, Station: Wilmington New Castle Co Airport, DE US 13781 and Local Climatological Data Hourly Observations October 2016).

Results

M. halterata flight activity in three different locations

Significantly more *M. halterata* adults (8.1 +/- 1.01 S.E.) were caught on the traps placed over turf areas nearest the mushroom houses. Very few flies were caught on the traps placed over spent mushroom compost piles (1.0 +/- 0.11 (S.E.) or near windbreaks (0.17 +/- 0.2 (S.E.), and the mean captures for these two locations did not differ significantly (Fig. 8).

Figure 8: Number of *M. halterata* flies (mean ± SE) captured on yellow sticky traps placed at multiple locations of the near surroundings of mushroom farms. Fly catches from two farms and three time periods over five days were pooled (n=30). Bars with different lettering denote significant differences among means (Tukey's Multiple Comparison Test, $P < 0.05$).

***M. halterata* flight activity during different time intervals, farms in August**

The results from the general linear model testing time interval, farm and farm by time interval are presented in table 2.

The number of phorid flies caught differed among the time intervals tested. More flies per hour (21.3 +/- 1.9 S.E.) were captured between 4 PM to 12 midnight than during the other two time intervals. The lower captures during the 12 AM-to-8 AM and 8 AM-to-4 PM time periods (12.2 +/- 1.2 S.E., and 9.9+/-1.3 S.E., respectively) did not differ from each other (Fig.9).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Time interval	11478.048	2	5739.024	48.277	**.000**
Farm	111475.104	3	37158.368	312.579	**.000**
Farm * Time Interval	13694.706	6	2282.451	19.200	**.000**
Error	55277.743	465	118.877		
Total	292870.480	477			

Adjusted R Squared = .706

Table 2: Main effects and interaction of time interval and farm sampled over the mean number of mushroom phorid flies per hour caught on traps in August. (GLM, $p < 0.05$).

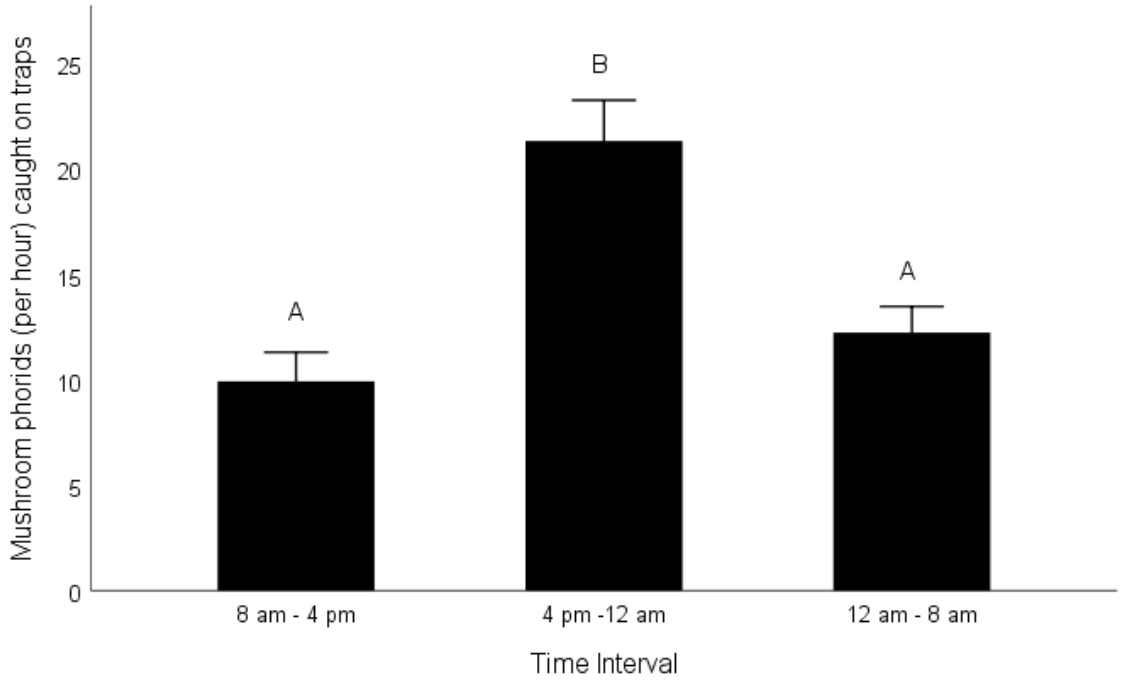

Figure 9: Number of *M. halterata* flies (mean ± SE) captured on yellow sticky traps over turf areas at three different time periods of the day during August, 2016. Fly catches of the two traps were pooled for each mushroom farm over five days (n = 60). Bars with different lettering denote significant differences among means (Tukey's Multiple Comparison Test, P >0.05).

Likewise, the number of phorid flies caught per hour differed between farms. Farm 4 had the highest number of captured of flies (40.2 ±2.0 S.E.) followed by farm 2 (10.9 ± 1.2 S.E.). Traps on farm 1 and 3 both had the least flies (0.4 ±0.3 S.E. and 6.5 ±0.3 S.E.) respectively (Fig. 10).

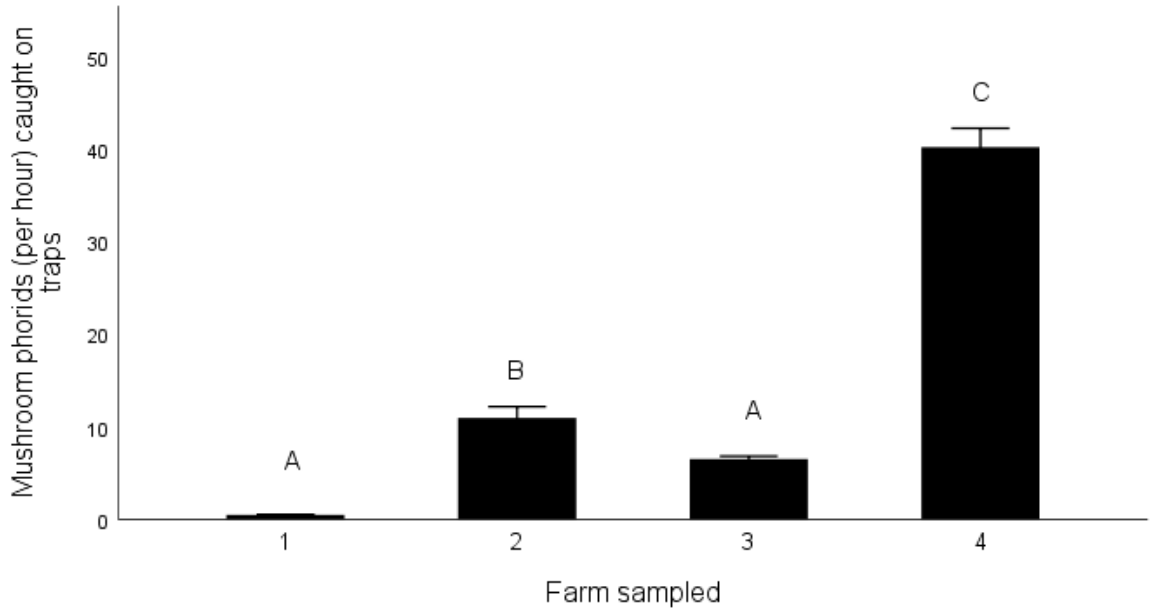

Figure 10: Number of *M. halterata* flies (mean ± SE) captured on yellow sticky traps over turf areas on the four mushroom farms during August. Fly catches from the two traps were pooled for each of the three time periods over five days (n = 60). Bars with different lettering denote significant differences among means (Tukey's Multiple Comparison Test, $P > 0.05$)

Phorid flies by time interval and farm

There was a significant interaction between time intervals and the farms sampled (Fig. 11). While farms 2 and 4 held the same time pattern, with most flies being active between 4 and 12 pm, farms 1 and 3 showed no time pattern.

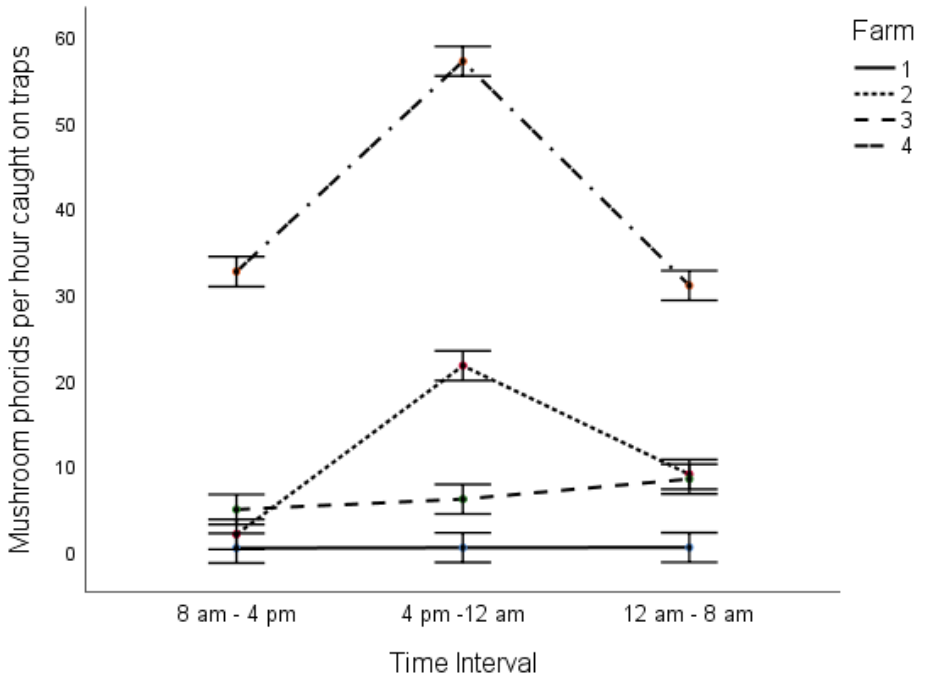

Figure 11: Number of *M. halterata* flies per farm and time interval in August 2016. Separate lines show the mean ± SE of *M. halterata* captured at each farm and time interval tested.

***M. halterata* flight activity during different time intervals and farms in October.**

The effect of time interval and farm sampled over the amount of phorid flies caught per hour in October is presented in Table 2. October diurnal flight patterns were similar to those of August except that night-time capture was drastically reduced. The greatest number of flies per hour was caught between 4 PM to 8 PM (61.4±15.5 S.E.). Low levels were registered after 8 PM until 6 AM with practically no flies captured during this interval (0.2 ± 0.03 S.E.). The daytime intervals, and 10 AM to 4 PM (29.2. ± 9.2 S.E. and 33.6 ± 14.3 S.E. respectively), did not differ from each other (Fig. 12).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Time Interval	92767.844	3	30922.615	248.007	**.000**
Farm	48735.865	3	16245.288	130.291	**.000**
Farm * Interval	35072.051	9	3896.895	31.254	**.000**
Error	18952.026	152	124.684		
Total	292870.480	168			

Adjusted R Squared = .883

Table 3: Main effects and interaction of time interval and farm sampled over the mean number of mushroom phorid flies per hour caught on traps in October. (GLM, $p < 0.05$).

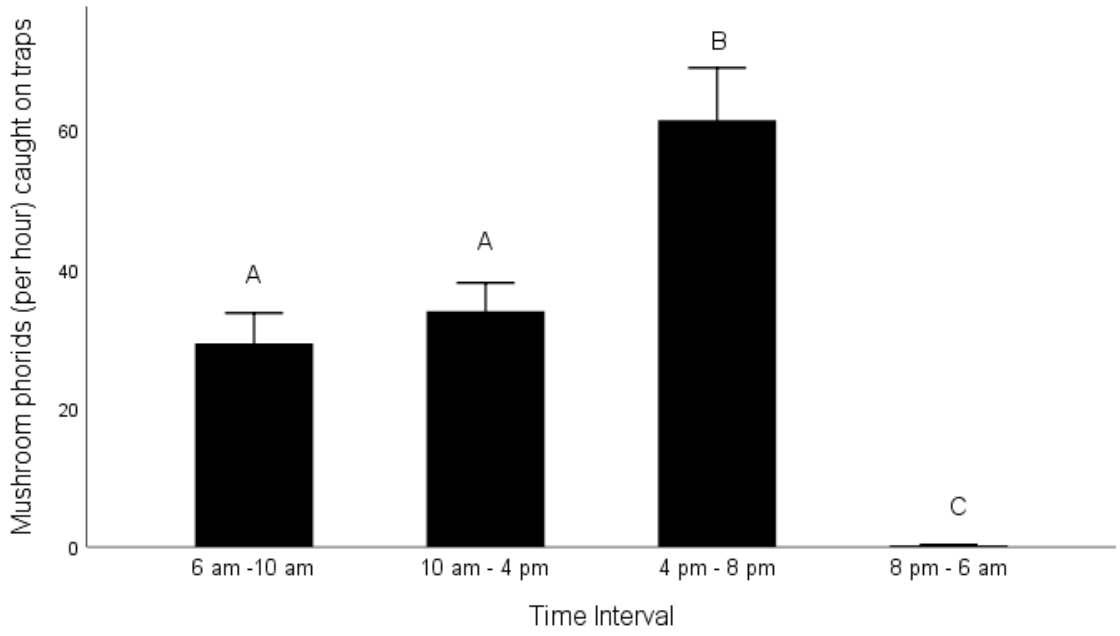

Figure 12: Number of phorid flies (mean ± SE) captured on yellow sticky traps placed over turf areas at four different time periods of the day during October, 2016. Fly catches of the two traps were pooled for each mushroom farm over three days (n= 24).

The numbers of flies captured on the four farms during October differed significantly, although this time, per-hour trap captures on all farms were much higher than in August. Farm 1 captured the fewest flies (5.6 ± 8.3 S.E) and traps on Farm 2 exhibited significantly higher capture levels than the other three farms (43.1. ±44.5 S.E.) (Fig. 13). Farms 3 and 4 captured intermediate levels of flies.

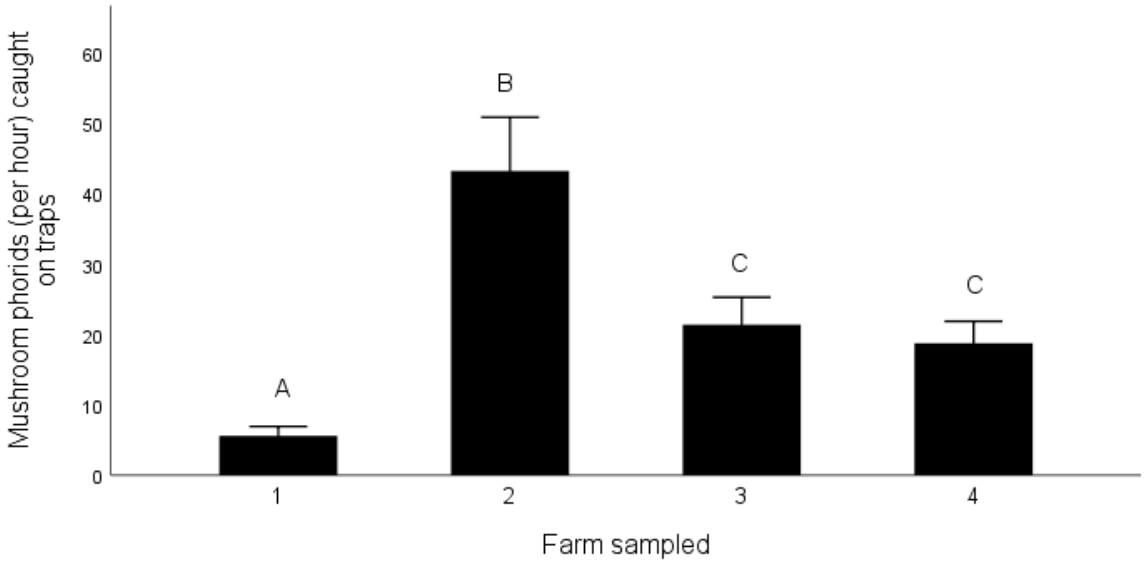

Figure 13: Mean number of phorid flies caught on sticky traps placed over turf areas on four farms during the October trapping experiments. Fly catch numbers of all time intervals pooled were pooled (N=24).

Phorid flies by time and farm in October

There was an interaction in the mean number of phorid flies per hour by time interval and farm. While farms 1, 2 and 3 showed a similar pattern to that of the main effects, farm 4 differed slightly. This farm had a higher phorid fly activity from 10 am to 8 pm (Fig. 14).

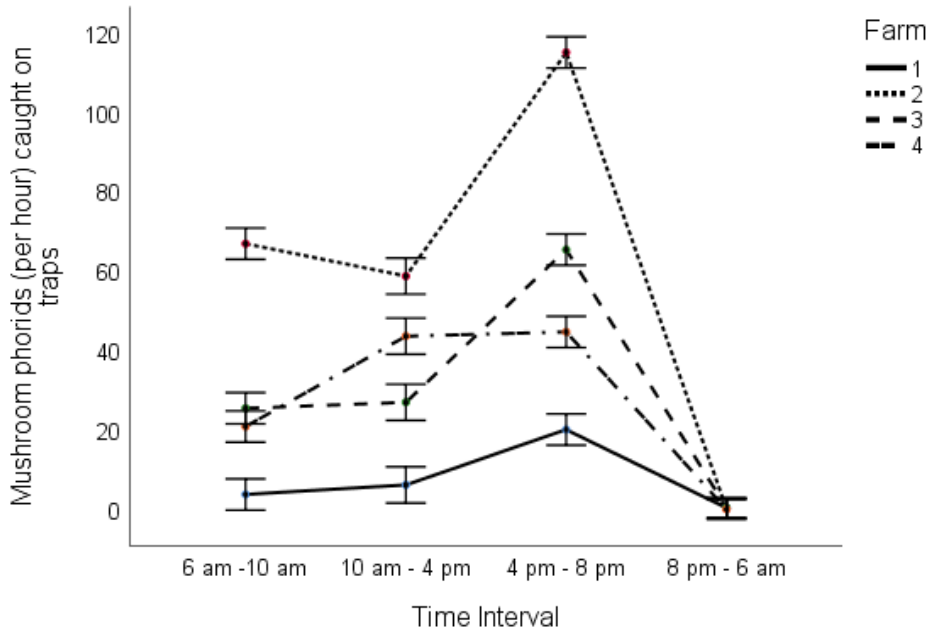

Figure 14: Number of *M. halterata* flies per farm and time interval in October 2016. Separate lines show the mean ± SE of *M. halterata* captured at each farm and time interval tested.

Sampling of adults flying over turf for evidence of sexual activity

On both nights in October phorid flies began flying in great numbers over turf around 5:00 PM and this activity lasted until sunset, which occurred ca. 7:00 PM. After the sun set all adult flight activity ceased over the turf areas we were observing. These assemblages of thousands of flies cruising at high speeds over grass were seen primarily at altitudes between 0.1 – 2 m over the same turf areas upon which the yellow sticky traps had been placed. Some flies appeared to achieve copulation aerially and pairs *in copula* could easily be observed in flight (Fig.15) compared to singly flying, non-copulating

adults. We estimated on both nights that roughly 30% of the flying adults were flying in copula within these turf areas at any given time.

Figure 15: Male and female *M. halterata in copula* on the hood of a car parked near the grassy turf area where thousands of adults were sweep-netted in our study. Approximately 30% of the aerially sweep-netted flies were collected *in copula*.

Turf samples for immatures and adult emergence

Although we captured the greatest numbers of *M. halterata* over turf areas close to mushroom houses we found no evidence that fly larvae were developing in the soil. Out of 12 samples taken during the heavy flight, 0 adults emerged from all the soil/turf

samples over four weeks, and not a single egg, larva, or pupa was found in any of the dissected subsamples.

Discussion

The very low number of *M. halterata* fly catches on the sticky cards placed over spent mushroom compost piles in August indicates that this substrate is not attractive to mushroom phorid flies nor is it a source of adult emergence, and hence not a source of re-infestation of mushroom houses. Our results are in agreement with Hussey (1965), who caught no female mushroom phorid flies on traps placed over spent mushroom piles in England. Previous studies have shown that *M. halterata* is an obligate fungal feeder that will not complete its development on a mushroom substrate lacking *A. bisporus* mycelia (Sheepmaker et al., 1996). Female *M. halterata* have been shown to be attracted to mushroom compost that has actively growing *A. bisporus* mushroom mycelia (Baker et al., 1982; Pfeil & Mumma, 1993; Tibbles et al., 2005) and hence will be most likely to enter mushroom growing rooms at the middle and end of spawn run (stage of active mycelial growth) of the crop (Richardson & Hesling , 1978). Steaming the mushroom compost at the end of the crop is a common agricultural practice employed in mushroom farming (Beyer, 2002). This procedure eliminates any pests or diseases remaining in the compost as well as eliminating mushroom fly immature instars. In most cases only after this steaming process do farmers empty the production houses. At such high temperatures the *A. bisporus* mycelia that is present in the compost is killed, which should render the

compost unattractive to *M. halterata* flies. The negligible number of *M. halterata* flies that we caught over spent mushroom compost piles supports this idea, and the few flies captured were most likely those flying around the general vicinity of the mushroom houses.

The low number of flies caught on the sticky traps near windbreaks could simply mean that *M. halterata* adults do not normally inhabit wooded areas even though many fungal species are present in the leaf litter, and the flies prefer to concentrate their activities over turf areas. Alternatively, flies emigrating out of the houses do not or cannot reach areas farther from the mushroom houses. All of the turf areas were at distances of 15 to 52 m from the mushroom houses, and so it seems likely that these data from the grass near the mushroom houses vs. farther away near windbreaks would mean that the flies captured in these locations originated from within the mushroom houses. Life histories and distribution of most phorid species in nature are unknown. *Megaselia halterata* often has been reported in association with the occurrence of mushroom farms (Disney, 2006; Brown & Hartop, 2017), rather than with natural areas of any kind.

Flies observed flying over the grassy areas and the sweep-netting of thousands of these flies strongly indicates that these grassy areas are sites for *M. halterata* courtship and mating. Approximately 30% of the flies captured in aerial flight via sweep-netting were *in copula*. We observed many *M. haltera* in courtship pursuits on the hood of our car or in copula there (Figure 8). These findings are new evidence that *M. halterata* leave the mushroom houses to find mates during daylight. The fact that we found zero *M. halterata* eggs, larvae, or any adult emergence from our turf samples taken over several areas where we had trapped flies indicates that *M. halterata* are not using the grassy turf

for egg laying. Rather, the aggregations of flies over turf seem to be predominantly for the purpose of mating. The females therefore must be flying somewhere else for oviposition, and this means they likely return to the mushroom houses to do so. Swarming behaviors in the family Phoridae have been reported, including *M. halterata* and other *Megaselia* species (Coyler, 1954). Hussey (1965) reported cases in which *M. halterata* were found outside mushroom houses and suggested that female flies leave the mushroom growing rooms in response to a pheromone-based stimulus from males. Although we did not record the sex ratio of the flies caught over turf areas outside mushrooms houses, our data suggests that flies are exiting the growing houses, concentrating over turf areas and mating. Mushroom production is an intensive farming system, where while one growing house is in the final stages of production, another house might be in the initial stages of the crop (stage of active growing *A. bisporus* mycelia in the compost). It is likely that the flies are re-entering these houses and infesting the compost at these earlier stages.

In August as in October the greatest number of flies were caught after 4 pm. In August, these data correspond to an interval from 4 PM to 12 AM EDT, where temperatures ranged between 27 and 17 °C, respectively. Sunset during this sampling period was at approximately 8 pm. According to Hussey (1965), *M. halterata* activity is curtailed by sunset, even if the temperature remains above the critical threshold for flight of 12.8 °C. We hypothesize that the flies caught during this August interval were mostly likely active before sunset, when temperatures before that hour were above 20 °C. In October, the high level of phorid fly activity was from 4 PM to 8PM EST. This interval had temperatures ranging between 23 and 15 °C. Even though temperatures were above

the critical flight threshold stated by Hussey (1965) for most part of this time interval, sunset was at approximately 7 PM, suggesting that flight was possibly curtailed by darkness even when temperatures should have been within a range conducive to fly activity for at least a portion of that time interval.

Nighttime fly activity was also low in both sampling occasions. In August the lowest numbers of flies were caught from 12 AM to 8 AM while in October, no flies were caught between 8 PM to 6 AM. Temperatures during the corresponding time interval in August ranged from 18-26°C, as opposed to lower night time temperatures in October which ranged from 17 -11. ° C. The negligible fly numbers caught in October during the night hours as opposed to August could be due to these lower nighttime temperatures.

Both sampling periods had intermediate levels of phorid fly activity during the day (8 AM to 4PM in August and 6 AM to 4 PM in October), where temperatures ranged from 21 and 32 °C in August and 13 and 23. °C in October. There has been no mention of an upper temperature threshold for *M. halterata* activity, but our data suggests that the flight activity of mushroom phorid flies in August may have been diminished by temperatures higher than 27 °C. As of October, more flies were caught in morning hours than in August, which suggests that flies were active during the day due to cooler temperatures. According to Hussey (1965), mushroom phorid fly activity does not become 'general' until temperatures exceed 15.5 °C. In October, this 'general activity' temperature did not occur until 8 AM, which alternatively suggests that the low mushroom phorid catches during this interval were due to low captures between 6 and 8 AM, when temperatures were not within the range favoring flight activity. Our

temperature data was taken from a historical, airport station data base, which may not reflect exact temperature values on the farms, perhaps further sampling experiments with on-site temperature measurements may elucidate even further the relationship between *M. halterata* activity, daylight and temperature.

Establishing temperature and daylight thresholds for *M. halterata* flight activity may be useful in developing IPM tactics for this species. The most successful IPM tool that mushroom growers use at present is fly exclusion. There are three main stages in the mushroom crop cycle when exclusion is hindered due to the opening of the mushroom house doors for long periods of time: the day the mushroom house is filled or spawned; the day the compost is cased; and during the harvesting period. During these stages, the crop is susceptible to mushroom phorid fly invasions, which can be reduced by scheduling these activities during hours when outdoor mushroom phorid flight is minimal. Our data suggest that farm operations should be limited to nighttime hours possibly after sunset and should be avoided after 4 PM until sunset in order to enhance fly exclusion measures. Further studies are needed to determine with far more precision the activity of *M. halterata* in relation to daylight and temperature.

Assuming the mushroom phorid flies caught on the traps over the turf areas came from mushroom farms, contrasting fly captures between the farms sampled may be due to varying infestation levels of *M. halterata* within the farms. We do not know exactly which variables related to a farm's location nor which farm management practices influence phorid fly density. There are many types of farms in Chester County, PA. They differ in size (number of growing rooms), construction material (block and metal), age (old constructions and new constructions), and management (organic, non-organic).

Finally, in Chester County there are multiple farms. Some farms are closer to each other than others (a distance of a couple meters to 1 km), while other farms are more isolated (no neighboring farms within at least 1 km radius). All the above factors have been suspected to influence the level of phorid fly infestation on a given farm.

There are many contrasting factors that may be related to phorid fly incidence in this particular study. For example, farm 1 had the lowest number of flies on both sampling occasions. This farm is large (consisting of 22 growing rooms), it is organically managed and it is isolated from other mushroom farms (it does not neighbor with any mushroom farm within a 1 km radius), which renders the farm unsusceptible to fly invasions from other farms. Farm 4 had the largest numbers of flies in August, this farm is small (it has 10 growing rooms), it is also organically managed yet it is not isolated from other mushroom farms (neighboring with 5 mushroom farms within a 1 km radius). Farm 2, which had the largest amount in October, is large (24 growing rooms), it is isolated and has a ‘’Dutch” style construction, which is suspected to provide better exclusion of flies.

We cannot explain the difference in phorid flies between farms with our data. Perhaps a larger data set would elucidate how all these factors, related to farm characteristics, influence levels of fly infestation.

It is interesting to note the time by farm interactions, particularly for farm 4 in October, which deviated from the overall time pattern, with the same number of flies from 10 AM to 8 PM. Perhaps a certain farm operation in early morning hours could have influenced this trend. However, determining to what extent the farming activities performed at certain stages in the mushroom growing process affect the number of phorid

flies outside the farms is difficult. The activities that might influence phorid fly density outside mushroom farms are casing and harvesting. When the casing material is brought inside a growing room, the doors remain open for approximately four hours. At this stage of the crop, there tends to be a high phorid fly infestation caused by those flies that have invaded the room to oviposit on the compost surface. One theory is that during casing, flies inside a room might migrate to the outside, attracted by the light when doors are opened. The other farm activity that can affect outside fly density is cropping or picking. When the crop is being harvested, the first generation of phorid flies is emerging and populations are also very high in the growing room. During cropping doors are open for most of the day.

Conclusions

Our study showed that mushroom phorid flies are indeed active outside mushroom farms for a crucial part of their lifecycle; that of mating. This stage of the mushroom pest's life history can potentially be interrupted in a mushroom IPM program, targeting flies that leave the mushroom houses to mate with the use of premise sprays or pheromone mating disruption, hence reducing the pressure from flies that re-enter the growing rooms to deposit eggs on the spawned compost.

Further studies are needed in order to determine *M. halterata* critical flight times, especially with regard to this species' flight activity in relation to crop stages so it can be determined when it is most likely that flies will emigrate from the growing rooms to the outside.

Chapter 5

Mushroom phorid fly (*Megaselia halterata*) population dynamics and risk factors associated with fly densities on commercial mushroom farms

Maria Mazin, Nina E. Jenkins, Thomas C. Baker, Edwin G. Rajotte

Abstract

Pennsylvania is the heart of the US mushroom industry, producing nearly 2/3 of the nation's mushrooms. The mushroom phorid fly, *Megaselia halterata* is a key pest in mushroom farming in most parts of the world. Mushroom phorid flies were monitored on 16 commercial mushroom farms in Chester County, PA from August 2016 to August 2017 with the use of light traps. Fly populations began in August, reaching their peak in November and declined drastically during the winter and early spring months. Population dynamics within the mushroom crops reveal that flies can invade as early as the first day of spawn run until the stage of casehold. In some crops, emergence was seen as early as 22 days after filling the growing room. In other crops emergence was seen between days 35 and 40 after filling. In some cases an overlap of fly generations occurred within the same crop and in other cases two generations occurred. A linear regression showed that factors such as the distance to neighboring mushroom farms and the number of neighboring farms as well as type of farm construction and steaming practices positively affected phorid fly densities on the farms sampled.

Introduction

Pennsylvania is the heart of the US mushroom industry, producing nearly 2/3 of the nation's mushrooms (USDA, 2014). The mushroom phorid fly, *Megaselia halterata* is a key pest in mushroom farming in most parts of the world (Richardson & Hesling, 1978; Keil, 2002). This species is an obligate fungal feeder and gravid females are highly attracted to mushroom compost with actively growing mushroom mycelia (Kielbasa & Snetsinger, 1981; Tibbles et al., 2005). *M. halterata* typically enters mushroom growing rooms at spawn run (stage of active mycelial growth) and lay eggs on the mushroom mycelia, which the neonates feed on (Keil 2002). Studies on *M. halterata* are limited and have been done mainly in laboratory settings. Until c.a. 4 years ago, mushroom phorid flies were considered secondary pests and not a major concern, but recent outbreaks on commercial mushroom farms in Pennsylvania, in part due to changes in pesticide availability, have drawn attention to this species.

Implementing an integrated pest management (IPM) program requires understanding mushroom phorid fly population dynamics within commercial mushroom crops. In addition, the degree of phorid fly infestation varies from farm to farm and the factors affecting phorid fly density on a particular farm are unknown. Mushroom farms vary in management practices, which may have an effect on fly density, and anecdotal reports from mushroom growers also point to the influence of neighboring farms on phorid fly population levels. That is, a mushroom farm with many other neighboring mushroom farms may have a higher 'fly pressure' and therefore, higher levels of fly infestation than a more isolated farm. Since mushroom farms are densely concentrated in

the Chester County PA, the latter, if true, would emphasize the benefit of a collective effort to manage fly populations.

The mushroom production cycle can be summarized in the following steps: 1). Compost preparation; raw bulk materials are composted for the preparation of the mushroom growing substrate. Composting consists of two phases. Phase I composting consists of the initial breakdown of the raw materials. During phase 2 composting, the materials are pasteurized in order to kill any harmful organisms that can be detrimental to the A. bisporus. Phase 2 composting can be done in a tunnel (isolated chamber) or in the mushroom growing room itself. If phase 2 is done in a tunnel, the compost is moved to the growing room after pasteurization, called filling phase II compost. When phase 2 composting is done inside the growing room, growers fill the room with phase 1 compost and then pasteurize inside the growing room. (It is worth mentioning that growers may fill phase 3 mushroom compost, which consists of filling the growing room with fully spawned compost, meaning that the growing cycle within the room begins with casehold for which the growing cycle is much shorter) 2). Spawn run; after the compost cools spawn is added and the growth of A. bisporus mycelia begins and lasts for 14 to 16 days. 3). Case hold; once the mycelia have completely colonized the compost, a casing layer consisting of sphagnum peat moss is added over the top of the compost, and the A. bisporus moves from a vegetative to a reproductive state. The casehold stage lasts 14-17 days. 4). Production or cropping; the final stage of the growing process where the mushrooms fruits are fully formed. Mushroom harvesting is done in the first 2-3 days of a 7 day cycle called a flush or a break. Once mushrooms from a first break are harvested, the next flush of mushrooms begin to mature. Typically, production ends after two or

three breaks, depending on the commercial needs of the grower and on the quality of the crop. The production is ended when the growing room and compost are steam pasteurized. The pasteurized compost is removed and discarded to waste piles outside of the growing buildings, and the growing room is thoroughly sanitized in preparation for the next crop (Beyer, 2003; Pecchia & Beyer, 2013) Some growers steam pasteurize the growing room once it is emptied, called double steaming or steaming empty.

This process can be carried out in conventional mushroom farms, consisting of cement block rooms built on an incline. These farms contain multiple growing rooms separated by a cement block wall. Each growing room has to ends, with one door pointing to a breezeway that is for farmworker traffic. The other door, located at the opposite end of the growing room opens to a cement wharf and is utilized for operations such as filling or emptying the rooms and applying the casing layer. The other type of farms in which mushrooms are grown are called Ducth style farms. These farms, much newer than the conventional ones are made of aluminum walls and also have two ends similar to conventional farms.

Objectives

The objectives of this study were to characterize *M. halterata* populations within commercial mushroom crops and to determine whether certain factors related to farm location, such as the proximity to neighboring mushroom farms and the number of mushroom farms in a 0.5 km radius as well as farming practices such as type of compost filled, organic or non-organic growing practices, type of construction of the farm and

double steaming practices influence fly population densities within commercial mushroom farms.

Methods

Commercial mushroom farms

The study was conducted on 16 different commercial mushroom farms (recruited on a volunteer basis), all farms were located in Chester County, PA. Farming practices such as organic or non-organic growing, type of compost filled, and whether the farm double steamed as well as the type of farm construction (conventional or dutch) were recorded for each farm.

Fly monitoring and counting

Mushroom phorid flies were monitored with The Pennsylvania Fly Monitor (Barber, 2018). This monitoring device is a fluorescent, 15 Watt, black light (45 cm long), fastened to a board with a strip of white paper sprayed with Tangle-Trap ® on either side of the light. In this study we provided the growers with 17 x 4 inch white cards prayed with tangle foot (the card material being thicker than the paper normally used on commercial mushroom farms). One growing room from each mushroom farm was monitored. The same room was monitored throughout the entire 12-month study. The fly monitor was placed at the front end of the room near the access door (the breezeway end). As soon as the growing room was filled with the mushroom growing compost

substrate (phase I or phase II compost), monitors where introduced into the rooms and the white sticky cards were replaced with new ones every other day. For each card, the date of introduction and removal as well as the corresponding stage of the crop was recorded. After removing the cards from the monitor, the sticky side was covered in plastic 'Saran Wrap' and stored on the farm. The cards from all farms were collected on a monthly basis. This monitoring took place from August 1st 2016 to August 1 2017.

Flies on the sticky cards were counted using a camera and Image J, a public domain image processor (www.imagej.net) software. The software was calibrated by taking a random sample of 60 cards collected within the first three months of the study manually counting the flies on each card. Those counts where then compared to the counts generated by the software and a mean percent error (between the manual count and the software count) was computed, these data are summarized below (table 4).

Number of *M. halterata* on cards	**Mean percent error**	**S.D.**
0-500	0.13	0.6
500-1000	0.39	3.3
1000-2000	1.33	3.1
< 2000	8.49	15.9

Table 4: Mean percent errors and S.D. of *M.halterata* counts on fly cards with Image J ®

The cards with over 2000 flies were not accurately counted by the software, so we developed a sampling scheme in which 20 % of the card was counted manually (a

random number of 20, 2 x 2 cm squares on a grid placed over the card) which generated an error of 0.06 % +/- 4.3 S.D (N=30 cards).

Mushroom fly population dynamics within commercial mushroom farms

We used the fly card data from August 2016 through July 2017 to characterize phorid fly populations within commercial mushroom crops and populations throughout the year. The number of flies on each card per day of exposure for each month of the study as well as the crop stage was recorded for each participating farm. Fly counts were used to characterize mushroom fly population dynamics within mushroom production cycles.

Factors affecting phorid fly density (Fly pressure and farm management practices)

The following variables were taken into consideration as factors affecting phorid fly density among the farms sampled:

a) Number of neighboring mushroom farms within a 0.5 km radius from each participating farm. This was calculated using the radius function in Google Earth Pro (Google Inc.). A 0.5 km radius was drawn around the areal image of each farm and the number of neighboring mushroom farms within that radius were counted.

b) Distance of each farm to the nearest mushroom farm. This was calculated using the ruler function on the areal image of each farm on Google Earth Pro (Google

Inc.). The distance to the nearest farm was automatically calculated by the software and recorded.

c) Farm construction type: Conventional (farm built with cement blocks and wooden beds) or Dutch (farm built with aluminum walls and beds).

d) Type of Compost used to fill the house: Phase I or Phase II compost.

e) Double steaming practices: Whether the farm does or does not steam the growing room once the spent mushroom compost has been removed from the room.

f) Type of certification: Whether the farm is certified organic or non-organic

Statistical Analysis

We used linear regression analysis (SPSS v.24) against the log transformed phorid fly counts to test whether the variables listed above affected the number of flies on the commercial mushroom farms sampled. Given that during winter and early spring (January-May) phorid fly populations were very low, only the data from August-December of 2016 were used for the regression analysis.

Results

Mushroom phorid fly population dynamics within commercial mushroom farms

Data from 22 crops (August 2016-July 2017) suggest that phorid flies begin to invade the growing rooms from the first days of spawn run. Fly numbers increase during case hold and reach their highest level at the production phase (Fig.16).

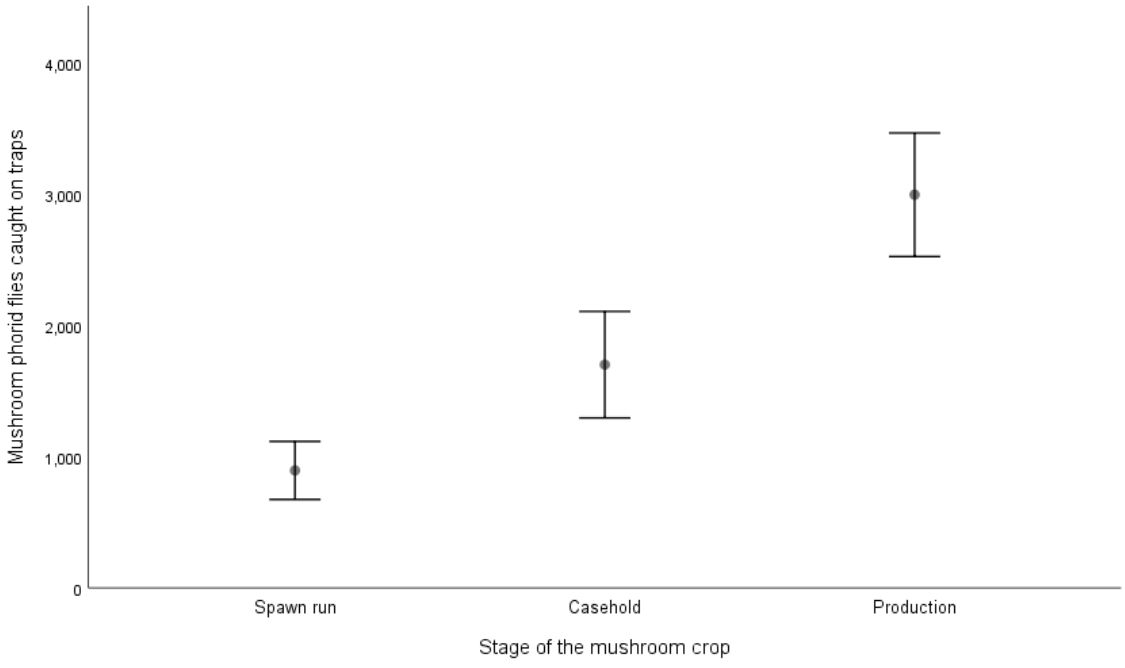

Figure 16: Mean (+/- 1 S.E.) *M. halterata* flies as a function of the stage of the crop. Daily fly numbers from 22 crops sampled within 16 farms from August -November 2016 were pooled.

Phorid flies began appearing on the fly monitors around day 2 after filling or spawning the growing rooms (irrespective of the type of compost being filled) and continued to invade the growing rooms throughout spawn run (Figs. 17, 18 & 19). We identified three major trends in population cycles within the mushroom crops sampled. 1). An initial invasion during spawn run (days 0-17) with a strong peak in fly numbers between days 35-45 after spawning or filling the growing room (Fig 17). 2). An initial invasion during spawn run and the beginning of case hold (days 0-20), a peak in fly numbers between days 22-25 and another peak between days 35-45 (Fig. 18). 3). An initial invasion during spawn run and the beginning of case hold and various random peaks in fly numbers between days 22-55 of the growing cycle (Fig. 19).

Ten crops out of 26 crops sampled also showed a peak in fly numbers between days 15-18 after filling or spawning the room, corresponding to the day in which the crop was cased.

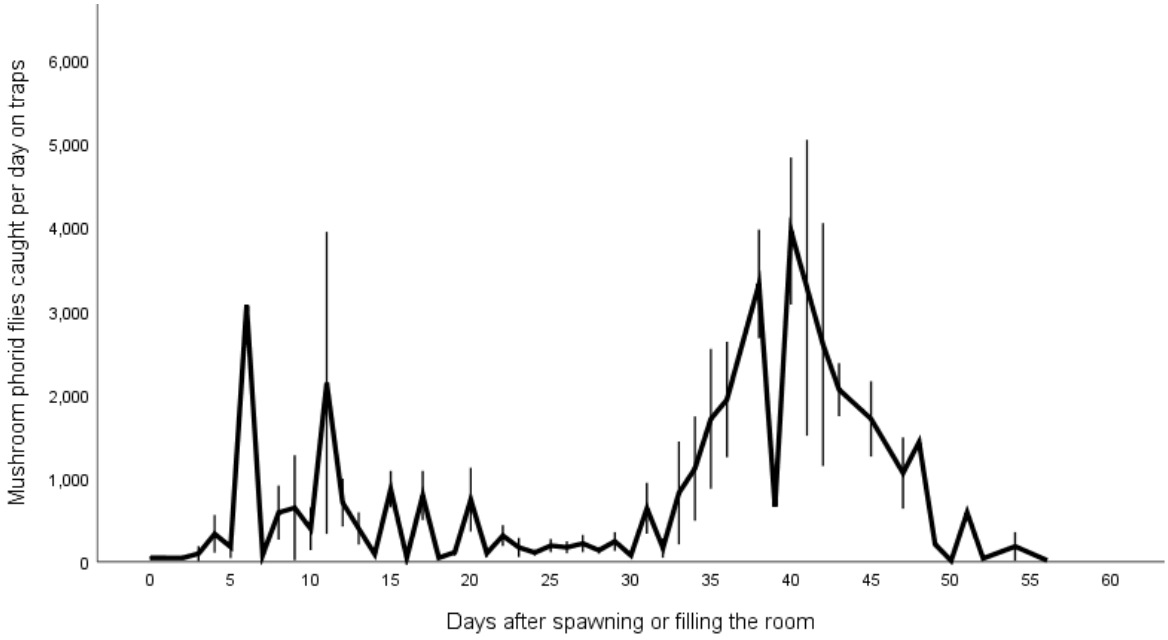

Figure 17: Mean number of mushroom flies per day as a function of days after filling the growing room. Y axis represents the mean +/- 1 S.E. This trend where there is an initial fly invasion during spawn run and casehold (days 0-30) and a peak in fly numbers at production (days 30-45) was seen in 10 of the crops sampled. Fly numbers per day from 10 mushroom cycles were averaged (N=10).

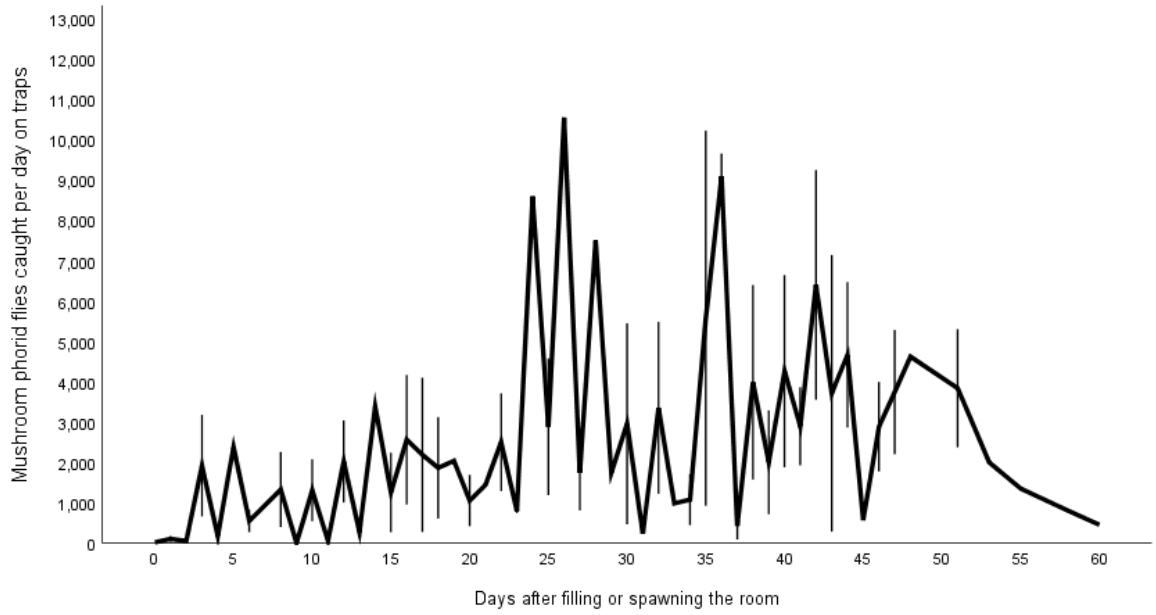

Figure 18: Mean number of mushroom flies per day as a function of days after filling the growing room. Y axis represents the mean +/- 1 S.E. This trend where there is an initial fly invasion during spawn run and casehold and a peak between days 22-28 followed by a second peak between days 35-45 was seen in 8 of the crops sampled (N=8).

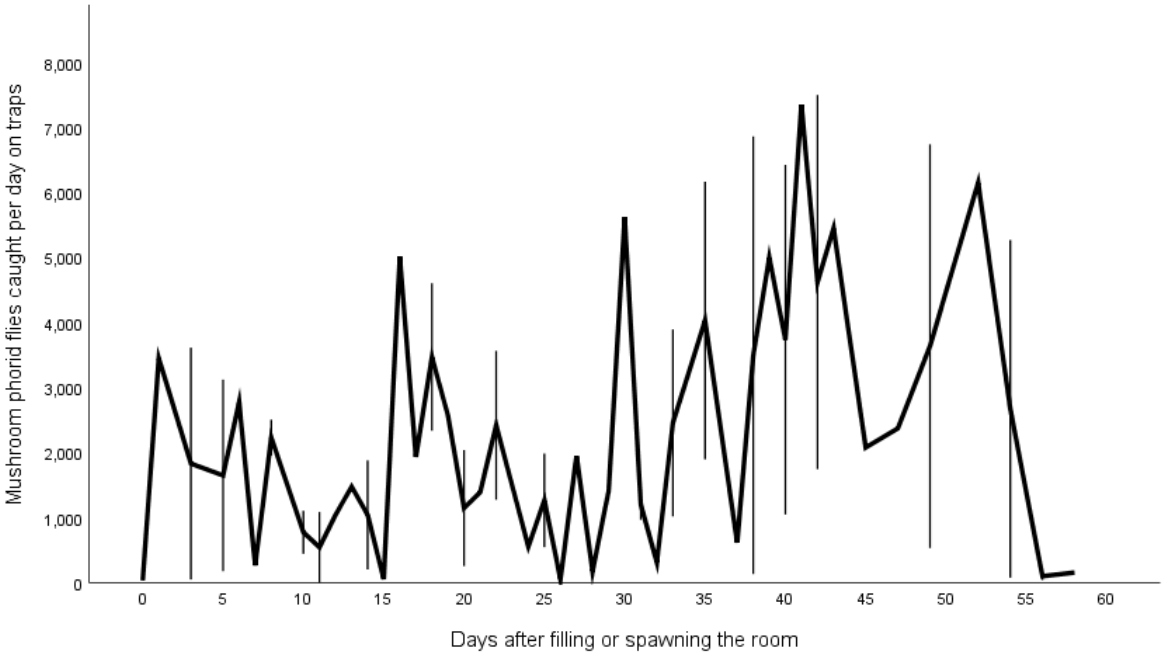

Figure 19: Mean number of mushroom flies per day as a function of days after filling the growing room. Y axis represents the mean +/- 1 S.E. This trend where there is an initial fly invasion during spawn run and followed by random peaks was seen in 4 of the crops sampled (N=4).

Megaselia halterata populations within mushroom farms nearly doubled from August/September 2016 to October/November 2016 and dramatically declined during the winter and early spring months (December-May) (fig. 20). Populations then began to rise again in June 2017. However, on some farms (5 out of 14), winter populations remained constant at low numbers (fig. 20).

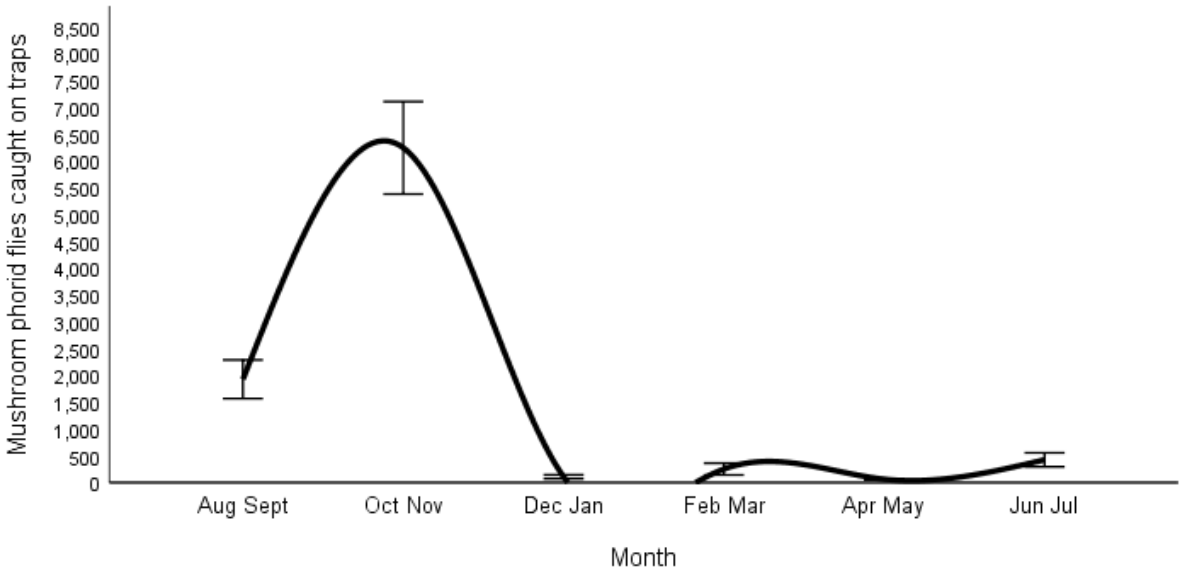

Figure 20: Mushroom phorid flies (mean +/- 1 S.E.) by month of the year August 2016- July 2017. Daily fly numbers on the traps for 34 crop cycles from 16 mushroom farms were pooled.

Factors affecting *M. halterata* density (Farm pressure and crop management practices)

The linear regression results are summarized in Table 5. The variables that were significantly associated with high mushroom phorid fly densities were, the number of neighboring mushroom farms in a 0.5 km radius, and the distance to the nearest mushroom farm. Furthermore, Dutch farms, and farms that do not steam the growing room once it is emptied had lower fly densities than conventional farms and those farms that do steam the growing room once emptied. Mean room air temperatures, type of compost and whether the farms are under organic production were not correlated to fly densities between farms.

	Unstandarized B	Coefficients S.E.	Coefficients B	t	Sig.
Constant	1.935	.116		**16.69**	**.000**
Distance to the nearest mushroom farm	0.000	0.000	.305	**4.33**	**.000**
Number of mushroom farms within 0.5 km	0.153	0.030	.364	**5.15**	**.000**
Does not steam empty	0.491	.107	.764	**4.5**	**.000**
Dutch construction	-0.687	.125	-.314	**-5.4**	**.000**

Table 5: Factors affecting *M.halterata* fly populations on commercial mushroom farms.

Discussion

M. halterata individuals begin to invade the growing rooms as soon as day 1 of spawn run and can continue to invade until casing, between days 14-17 after filling or spawning the room. Bins (1979) found a similar trend regarding the timing of fly invasion on mushroom farms in England. Strong peaks in fly numbers were mostly recorded between days 23 and 48 of the growing cycle, although fly numbers continued to increase if the growing cycle was extended to days 50-60 after spawning or filling the room. We hypothesize that these peaks (days 23 to 48) correspond to the emerging first generation of flies, the day of the peaks would depend on which day of spawn run and case hold the initial fly invasion was. Average spawn run compost temperatures vary depending on compost depth and the day of spawn run or casing. Anecdotal evidence

from mushroom growers in PA regarding the distribution of larvae and pupae point to a concentration of immature stages within the first 10 cm of the compost. According to this gradient, temperatures during spawn run vary between 27 C° at 10 cm, 25 C° at 5 cm and 21 C° at 2.5 cm from the compost surface (personal communication). During case hold, temperatures in the casing layer cool down to an average of 21 C°. It's been reported that development time from egg to adult of *M. halterata* is 20 days at 27 C°, 22 days at 25 C° and 24 days at 22 C° (Hussey, 1960; Barzegar et al., 2016). Our data shows that flies can emerge between days 22-25 after spawning or filling the room. In this case, we can assume that this early fly emergence corresponds to the progeny of female flies that laid eggs the very first days of spawn run. Fly emergence at days 35-45 would correspond to invading flies in mid to late spawn run (days 10-15) or even the first days of case hold (days 15-20).

In cases where a fly emergence as early as day 22 (approximately a week into the case hold stage) often another peak in fly numbers between days 35-45 was also present. This most likely assumes an overlap of a first generation of *M. halterata* emerging at different periods of the cycle, rather than two subsequent generations emerging in the same crop. By day 22 of the farming cycle, compost temperatures are much lower and *M. halterata* development would take longer (c.a. 25 days), since these crops where terminated by day 45 and an emergence of a second generation might not have been possible.

For crops that go beyond day 45 (figure 19), our data suggests that an overlap of the first generation of flies as well as two separate generations of flies can very much occur within the same crop. This is in agreement with Rinker & Snetsinger (1984) who

reared *M. halterata* in small mushroom growing rooms and Rinker (1981) who surveyed *M. halterata* populations in Chester County PA.

The strong peaks in fly numbers observed between days 14-17 in some of the crops sampled might correspond to invading flies at casing, when farm doors are opened and remain so for c.a. 4 hours. Navarro et al. (2001) reported similar results regarding a strong peak at the time of casing while monitoring mushroom phorid fly populations in Europe and concluded that the peak was most likely from invading flies at the day of casing. Mushroom mycelial odors at this time of the crop are at their strongest due to the complete *A. bisporus* colonization of the compost, which may attract a higher number of flies to the compost or the growing room (Richardson & Hesling., 1978).The latter has not been proven and further experiments are needed to confirm the influx of M. halterata during the c.a. 4 hours that the farm doors are opened during casing. Another highly possible explanation for the peak in fly numbers during casing is caused by disturbance of the mushroom phorid flies that are already on the mushroom beds at the time of casing. After filling the room and spawning, the mushroom bed are covered with plastic, at the moment of casing this plastic is removed and the casing material is put onto the beds. These activity might disturb the flies causing them to fly into the light traps.

Our data suggests that phorid fly control should be emphasized from the first day of spawn run until at least the last day of casing. If the crop is taken beyond day 45, special attention should be directed at days 23-25, when a first generation might occur and emerging adults from this generation should be expected to occur between days 48-50 of the crop cycle. Emergence from generations invading during mid to late spawn run should be expected between days 30 to 40 after spawning or filling the room.

In case of a fly invasion at the day of casing, a separate generation of flies can very well emerge from this invasion between days 40 and 45 in addition to those emerging from the spawn run invading flies.

The winter population data indicates that M. halterata is able to continue its lifecycle within mushroom farms, despite low outdoor temperatures in which they would not survive. Fly numbers during the winter remained low and began to rise in June, suggesting that perhaps warmer outdoor temperatures influence the population growth rate of *M. halterata*. Mushroom phorid fly activity outside farms has been reported (Hussey, 1960; Mazin et al. unpublished) and suggests that *M. halterata* leave mushroom growing rooms due to the high population pressure inside rooms once first generations emerge. Mating behaviour outside growing rooms has been reported by these same authors, who suggest that the female flies re-infest growing rooms after mating outdoors. Ideal temperatures from August to November (when we see an increase in within farm fly populations) might enable the fly population to reproduce at a faster rate.

Among the farm characteristics associated with *M. halterata* densities among the farms sampled, the construction type was significantly related to *M. halterata* populations. Dutch style farms had lower fly numbers than conventional farms built with cement blocks. Dutch style mushroom farms are built with large single aluminum panels, which may provide better exclusion from flies. The material does not succumb to the changes in temperature like cement blocks do, which eventually crack and break. This way, Dutch style farms have less cracks and crevices through which flies can enter the growing rooms.

The distance (km) to the nearest neighbouring mushroom farm as well as the number of neighbouring mushroom farms in a 5 km radius was positively correlated with phorid fly density within the farms sampled. This finding suggests that flies disperse between farms, although they have been characterized as weak flyers (their common name scuttle fly refers to their walking rather than flying behavior). Farms in the Chester County area are densely concentrated for which distances may be short and attainable for flight, another possibility is that flies are dispersed between farms by wind.

Those farms that do not steam the growing room after it is emptied (all farms in this study steam the growing room before it is emptied), had significantly lower fly densities than those farms that did steam the room empty. We cannot explain this finding with our data, never the less, it proves that in terms of fly control, steaming the room empty does not seem to matter. However, this practice is essential for pathogen control in the case of growers who fill phase II compost (Beyer, 2003).

The type of compost used to fill the room was not a significant predictor of phorid fly density between farms. *M. halterata* depends on the presence of mushroom mycelia in the compost in order to survive (Kielbasa & Snetsinger, 1981). For this reason, type I compost will not render an attractive substrate to ovopositing *M. halterata* females until it is spawned. This occurs after the compost has been pasteurized inside the growing room. The mushroom growing cycle begins when both types of compost have been spawned, which explains why we did not find any differences in fly densities between composts.

Finally, there were no significant differences in fly densities between organic and inorganic farms. This points out that the chemical and bio control methods that were used on the farms within our sample may not be effectively suppressing fly populations. This

suggests a resistant *M. halterata* population and highlights the importance of finding alternative control methods (i.e. improving fly exclusion, resistant *A. bisporus* varieties) that may help control flies.

We conclude that fly control efforts should be focused from the first days of spawn run until the first days of case hold. Attention should be paid between days 23-25 in case an early fly emergence occurs. Coordinated, area wide control efforts are crucial to the success of mushroom phorid fly control. Further research is needed in order to determine exact compost temperatures along the compost strata and at different stages of the mushroom crop cycle. Research regarding the distribution of *M. halterata* larvae and pupae within the mushroom compost on commercial farms would render useful for predicting lifecycles based on differing compost temperatures. This study was observational, rather than completely randomized, for which inferences cannot be made to the entire population of mushroom farms in Chester County. However, our data represents and wide array of farm types and farming practices for which our findings may render useful for focusing future research efforts.

Chapter 6

Perceived control predicts mushroom farmworker integrated pest management behavior

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Abstract

Integrated pest management (IPM) is an approach to managing pests that focuses on preventing infestations, monitoring pests, and limiting the use of harmful pesticides. IPM techniques that mitigate the spread of mushroom pests and pathogens are used in mushroom farms in Pennsylvania and their success is dependent on the frequency and consistency with which they are implemented by farmers and farmworkers alike. The Health behavioral model (HBM) is often employed for the study of risk mitigating behaviors. In this study we used the HBM to determine factors that predict IPM behavior in mushroom farmworkers. We found that high perceptions of risk and control around the spread of pests and pathogens and not IPM knowledge, predict IPM behavior. We conclude that IPM interventions should not be limited to education on mushroom pests and pathogens and should develop ways to encourage farmworkers perceptions of control over mitigating pests and pathogens.

Introduction

Integrated pest management (IPM) is an approach to managing pests that focuses on preventing infestations, monitoring pests, and limiting the use of harmful pesticides ("What is IPM," n.d.). It takes into consideration and integrates all available pest control techniques that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment (FAO, 2012). IPM has been a part of environmental and health public policy in the U.S. for over 40 years. In 1993 the US Department of Agriculture (USDA) launched the National IPM Initiative, the goal of which was to have 75% of US crop acreage under IPM by 2000 (Ehler, 2006). In 2002, the USDA launched the National Road Map for IPM which among its objectives intended to increase nationwide communication and efficiency of IPM practices within agriculture. The priority being to develop alternative pest management tactics that have major economic benefits, while also protecting public health, agricultural workers, and the environment (USDA, 2013).

The benefits of implementing IPM not only apply to the economy of the farm but to the overall health of farmworkers through the reduced need for pesticides and ultimately, a reduced exposure to pesticides (Goodell et al., 2004). Studies have confirmed that exposure to pesticides can be dermal, oral, and respiratory and can occur through direct contact with pesticides during application, contact with pesticide residue on plants, upon entering a recently treated area or through drift from nearby application (Hoekstra et al.,1996; Reidy, 1992; Suratman et al., 2015) . Farmworkers' family

members may also be exposed to pesticide residues that are brought home on farmworkers' clothing, skin, and equipment (Coronado et al., 2006; Deziel et al., 2015; Lu et al., , 2000). Health effects of pesticide exposure result in a wide range of acute health effects, including, nausea, dizziness, vomiting, headaches, stomach pain, rashes, and eye problems (Moses, 1989;Arcury & Quandt, 2003). Chronic exposure to pesticides can also have effects on the neurological, respiratory, immune, and reproductive systems (Gonzalez-Alzaga et al., 2014; Kamel & Hoppin, 2004; Muñoz-Quezada et al., 2013; Swan & Sharpe, 2006).

The commercial mushroom farming industry in the U.S. applies IPM to control mushroom pests and diseases that may pose a risk to the crop in the form of yield and economic losses. As in any integrated pest management program, mushroom IPM relies heavily on pest and disease prevention. Preventative measures have the objective of discouraging the establishment and subsequent spread of pests and diseases within mushroom farms (Cliff, 2002; Coles & Barber, 2002). The success of these measures relies on the frequency and consistency with which they are implemented by farm owners and farm workers alike, given the proximity of the farmworkers with the crop while on the job.

Pennsylvania is the number one producer of edible mushrooms in North America. During 2016-2017, 1.2 billion dollars in sales were generated, mostly in the Chester County and Reading areas, where the mushroom farming industry is concentrated. Each year, approximately 45,000 to 50,000 migrant and seasonal farm workers are employed in Pennsylvania to assist in harvesting the Commonwealth’s fruit, vegetable, and mushroom crops(Cason et al., 2004) .

Despite the important role that mushroom farmworkers play in the success of mushroom pest and disease prevention (Pecchia & Beyer, 2013), the extent to which workers implement (IPM) preventative measures and what influences IPM implementation within mushroom farmworkers has not been studied. These measures include keeping farm doors shut after entering or exiting a growing room in order to exclude mushroom fly pests, avoiding areas on the mushroom growing bed that have a visible sign of a mushroom disease and wearing clean clothing every day in order to prevent the spread of the disease through farmworker movement from one part of the farm to another and through farmworker clothing (Cliff, 2002; Pecchia & Beyer, 2013). Human implemented, IPM prevention measures can be seen as risk mitigating behaviors (mitigating mushroom pest and pathogen risks) or even as health behaviors in terms of mitigating pesticide exposure. A common model utilized in the study of human health behaviors is the Health Belief Model (HBM) (Rosenstock, 1966), which seeks to assess how behavior is a function of a person's subjective appraisal of risk. The current HBM model focuses on six main variables predict health/risk behavior 1) Risk susceptibility, the belief that someone is at risk 2) Risk Severity, beliefs concerning the seriousness of the risk 3) Benefits to risk behavior, beliefs about the effectiveness of a risk mitigating behavior 4) Barriers to risk behavior, beliefs about the costs of a risk mitigating behavior 5) Self-efficacy or locus of control, the belief that one can/cannot take action to mitigate risks 6) Cues to action: Knowledge provided by education material or personal experience about the risk. In addition, it has been proposed that demographic factors such as age, ethnicity, socioeconomic status etc., may also influence risk and self-efficacy perceptions (Carpenter, 2010; Janz & Becker , 1984). According to the HBM, persons

must perceive themselves susceptible to risk before they will take action. This relationship is modified by self-efficacy, recognizing one's ability to control exposure to harm, and cues to action, such as knowledge and training (Arcury et al., 2002).

Behavioral research applying the HBM to farmworkers in the U.S. has been conducted mainly within the context of pesticide safety. Studies have focused on farmworker knowledge, beliefs and perceptions about pesticide exposure risk as well as farmworkers sense of control (self - efficacy) over taking risk mitigating action. Studies have looked at how variables within the HBM correlate to one another as well as how they influence health behavior. For example, McCauley(McCauley, Sticker, Bryan, Lasarev, & Scherer, 2002) et al reported that knowledge about the health risks of pesticides and risk mitigating behaviors had an effect on risk perceptions and safety behaviors among adolescent Latino farmworkers. On the contrary Cabrera and Leckie (2009) found that even though farmworkers in California showed high pesticide exposure risk perceptions and knowledge, they did not engage in pesticide safety behaviors. Arcury (2002) found that pesticide exposure knowledge among farmworkers in North Carolina was strongly related to perceived risk but not to safety behavior, while perceived control over avoiding harmful effects of pesticides and engaging in self-protective behaviors, was related to safety behavior.

In this study, we employed the HBM to asses mushroom farmworkers risk and control perceptions regarding mushroom pest and pathogens. Risk perceptions in the context of mushroom farming refer to perceived risk of mushroom pest and pathogen spread within and among mushroom farms. Control perceptions refer to the whether farmworkers perceive themselves to be in control of the spread of mushroom pests and

pathogens while working in addition to a sense of control over the performance of IPM measures. Specifically, we asked whether farmworkers risk and control perceptions, as well as their knowledge on IPM influence the frequency of engaging in IPM behaviors. IPM behaviors are defined as measures that must be put into practice on a daily basis in order to prevent the establishment and spread of mushroom pests and diseases. In addition, we hypothesized that certain farmworker characteristics such as gender, age, place of origin, occupation, type of pay, years working on mushroom farms, salary, education and English proficiency might also influence IPM behavior.

Methods

We developed a survey instrument designed to measure participants' risk and control perceptions around mushroom pests and pathogens, knowledge on IPM and the frequency with which they engaged in IPM behaviors (**Appendix A**). Data on farmworker characteristics (age, gender, income, years of education, years working in mushroom farms, type of pay, personal English proficiency and supervisors' English proficiency) was also collected. Perceived risk was measured by presenting participants with common situations regarding pests and pathogens and asking how much worry the situation caused them. For example: 'The doors to the mushroom farm are not closed and mushroom flies enter the room. The answers to these questions were: a).This is a problem that I don't worry about b) This is a problem I worry very little about c) This is a problem I worry moderately about d) This is a problem I worry a great deal about. Perceived control questions were aimed at measuring how much internal control participant's believed to have over the establishment and spread of mushroom pests and diseases and over engaging in IPM behaviors. For example 'How much control to you think you have over keeping mushroom

flies from spreading from one growing room to another?' The answers to which were: a) No control b) Small amount of control c) Moderate amount of control d) A great deal of control. IPM knowledge was measured using 6 agree-disagree statements developed by experts in the field about basic mushroom pest and pathogens, i.e. 'Mushroom diseases can stick to work tools'. The frequency of engaging in IPM behaviors was evaluated by mentioning mushroom IPM practices (which were selected by experts in the field) and inquiring about the frequency with which each behavior was performed. For example, "How often do you close the door when you enter/exit a growing room?" The answers to these questions were: a) 6-7 days a week b) 5-3 days a week c) 2-1 days a week d) zero days a week. Farmworkers characteristics were measured with multiple response questions or with continuous data responses where applicable.

The survey was developed and delivered in Spanish or English depending on the participants' preference. The interviewers were fluent in both languages and completed c.a. 4 hours of previous training where practice interviews were performed. Interviews were completed in approximately 25 min and performed in private spaces on mushroom farms in Berks and Chester Counties, PA, USA, after receiving authorization from the farms management personnel. Mushroom farmworkers who were harvestors, irrigation staff, mushroom disease control staff and farm managers were surveyed. The criteria for participant selection is based on the workers proximity with the crop and hence the importance of their participation in IPM, as well as the expectation from farm owners that they perform IPM behaviors. Participants were given information about the study and the interview and were asked for consent. The protocol for this study was approved by the Institutional Review Boards of the Pennsylvania State University.

Data analysis

Descriptive statistics including frequencies and percentages were generated for farmworker characteristics. Categorical scores for risk and control perception and IPM were created and measured on a scale of low, medium and high and low and high for the latter variable. IPM knowledge was categorized as low and high and IPM behaviors as low, medium and high. The association between risk and control perception, IPM knowledge, IPM training, farmworker characteristics, and IPM behavior was determined with a Chi square analysis. Subsequently, a stepwise logistic regression using those variables that resulted significant in the Chi square analysis was performed to determine how they predict the IPM behavior. All statistical analysis was performed in STATA. Data from a total of 105 surveys was used in the analysis.

Results

Farmworker characteristics

The mushroom farmworkers who participated in the study were mostly male and from Mexico (Table 6). Fourty four percent of the respondents had 1-8 years of formal education. The average number of years working in agriculture in the U.S was 16, 15 of which has been spent on mushroom farms. The majority of the sample population was paid by the hour or by the pound. Almost half (49.5 %) of the sample population worked as mushroom harvestors.

	Frequency or Mean	Percentage or SD
Gender		
Male	93	90.0
Female	10	9.2
Age		
Below 30	29	27.62
30-39	27	25.71
40-49	24	22.86
50 and over	25	23.81
Place of origin		
U.S.	11	9.2
Mexico	101	84.2
Other	6	5.0
Type of pay		
Hourly	51	49.04
By the pound	37	35.58
Combination	8	7.69
Salary	8	7.69
Income		
>20,00	13	12.38
29,000-29,999	25	23.81
<30,000 USD	52	49.52
I don't remember	15	14.29
Occupation		
Harvester	52	49.5
Irrigation Staff	15	14.3
Disease Spotter	11	10.5
Crop Manager	27	25.7
Years Agricultural Labor		
Total in US	16.32	12.22
Mushroom Industry	15.54	12.16
Current farm	11.17	10.75
Education		
<1 year	12	11.43
Grades 1-8	47	44.76
Grades 9-11	24	22.86
Grades 12 or GED	15	14.29
Any college	7	6.66
English Proficiency		
Little	35	33.65
Somewhat	41	39.42
Very well	28	26.92

Table 6: Mushroom farmworker characteristics (n=105)

Mushroom farmworker perceived risk, perceived control and IPM knowledge

Mushroom pest and pathogen perceived risk among mushroom farmworkers was in general high. Seventy per cent of the study participants had a high risk perception. Control perceptions were mainly divided between moderate (45 percent of the participants) and high (40 percent). Knowledge on IPM was almost equally divided between low knowledge and high knowledge (54 and 49 per cent, respectively (table 7).

	Frequency	Percentage
Perceived Risk		
Low	15	14.29
Moderate	16	15.24
High	74	70.48
Perceived Control		
Low	14	13.33
Moderate	48	45.71
High	43	40.95
IPM Knowledge		
Low	57	54.29
High	51	49.04

Table 7: Mushroom farmworker risk and control perceptions and IPM knowledge (n=105)

Associations between perceived risk, perceived control, IPM knowledge, farmworker characteristics and IPM behavior

Results from the chi square analyses are summarized in table 8. There was a significant association between perceived risk and IPM behavior ($\chi^2 = 8.82,\ p = 0.001$) and perceived control and IPM behavior ($\chi^2 = 12.2489,\ p = 0.016$). There was no association between IPM knowledge and IPM behavior ($\chi^2 = 2.43, p = 0.297$). Among

farmworker characteristics, income ($\chi^2 = 15.495, p = 0.050$) and position on the farm (x, p=0.042) were associated with IPM behavior.

IPM Behavior					
	High	**Moderate**	**Low**	***X²***	***p***
Perceived risk					
Low	4	4	6	8.82	0.001
Moderate	6	6	4		
High	43	23	7		
Perceived Control					
Low	3	4	6	12.24	0.016
Moderate	23	18	6		
High	27	11	5		
IPM Knowledge					
Low	25	19	13	4.56	0.102
High	28	14	4		
Gender					
Female	5	5	10	2.95	0.229
Male	48	28	93		
Age					
Below 30	15	5	8		
30-39	15	9	1	8.75	0.188
40-49	10	10	3		
50 and over	11	8	5		
Place of origin					
U.S.	5	2	1	3.22	0.521
Mexico	45	27	16		
Other	3	4	0		
Type of pay					
By the pound	14	13	10	7.36	0.297
Combination	5	2	1		
Salary	6	2	0		
Income					
<20,000	5	2	5		
20,000-29,999	11	8	6		
30,000-39,999	17	7	6	15.49	0.050
>40,000	13	7	0		
I don't know	7	8	0		
Position on farm					
Harvester	22	15	14		
Irrigation	6	7	2	13.05	0.042
Disease	8	2	0		
Manager	17	9	1		
Years on farm					
<10	14	5	5		
>=10	39	28	12	1.89	0.393

Self-Evaluated English					
Little	20	10	5		
Somewhat	16	17	7		
Very well	16	6	5		
Education					
No education	6	4	2		
Grades 1-8	24	15	8		
Grades 9-11	12	9	2	3.38	0.955
Grades 12/GED	7	3	4		
Any college	4	2	1		

Table 8: Associations between Farmworker Characteristics and IPM Behavior (n=105)

The results of the stepwise logistic regression are summarized in table 9. Model 1 of the stepwise logistic regression resulted in a significance of the effect of high risk perceptions over IPM behavior (OR 4.375, 95% C.I. 1.09-17.42 $p<0.05$)**.** In model 2 there was an association between high control perceptions and IPM behavior (OR, 4.452, 95% C.I. 1.14 -17.37, $p< 0.05$) yet risk perceptions resulted insignificant. In model 3 high control perceptions continued to be associated with IPM behavior (OR 10.341, 95% C.I. 1.89-56.37, $p<0.01$) as well the age interval of 30-39 years (OR 9.694, 95% C.I. 1.66-56.42, $p< 0.05$), an income of over 30,000 (OR 14.459, 95% C.I. 2.29-90.92, $p<0.05$) and the position on the farm corresponding to disease control (OR 35.881, 95% C.I. 1.34-956.14, $p<0.05$).

	Model 1	Model 2	Model 3
Perceived risk			
Low Risk	1	1	1
Moderate Risk	1.522	1.561	0.954
High Risk	**4.375***	3.092	3.054
Perceived Control			
Low Control		1	1
Moderate Control		2.591	3.399
High Control		**4.452***	**10.341****
IPM Knowledge			
Low Knowledge			1
High Knowledge			0.875
Place of Origin			
U.S. Born			1
Foreign Born			0.582
Type of pay			
By hour or salary			1
By yield or combination			1.802
Income			
<20,000			1
20,000-29,999			5.671
>30,000			**14.459****
I don't know			6.789
Position on farm			
Picker			1
Irrigation Staff			0.779
Disease Spotter			**35.881***
Crop Manager			3.94
Years Mushroom Farm work			
<5			1
5-9			0.225
>=10			0.263
Self-Evaluated English			
Little			1
Some			0.346
Well			1.432
Education			
Less than 8th Grade			1
9th-11th Grade			1.417
High school or GED			0.597
Some college			0.729
Cut 1			
Constant	0.59	1.24	2.891
Cut 2			
Constant	3.079	**6.973***	32.827

*p<0.05, **p<0.01, ***p<0.001

Table 9. Influence of perceived risk, perceived control and farmworker characteristics on farmworker IPM behavior

Discussion

Our results show that risk and control perceptions influence IPM behavior among mushroom farmworkers, while knowledge about IPM does not. According to the HBM, persons must perceive themselves susceptible to risk before they will take action. Furthermore, the HBM states that relationship between perceived risk and behavior is modified by an internal locus of control over risk mitigating action and cues to action, such as knowledge and training (Carpenter, 2010; Janz & Becker , 1984; Rosenstock et al., 1988).

The results from the stepwise regression we performed show that mushroom farmworkers have a high perceived risk around mushroom pests and pathogens, which begins to influence their IPM behavior, yet, it is a high perceived locus of control over preventing pests and pathogens and over implementing IPM that ultimately leads to IPM behavior. These results are in agreement with other studies among Hispanic farmworkers in the U.S. which have shown that more than perceived risk and knowledge, perceived control is often the strongest factor predicting pesticide exposure risk mitigating behavior (Vaughan, 1993;Grieshop et al., 1996; Austin et al., 2001; Edelson et al,, 2018). Some of these studies point to factors such as language barriers (difficulties communicating with the employer), time pressure on the job, (having to pick as much product possible in a limited amount of time) or logistics (such as not being able to effectively pick produce wearing bulky protective equipment) as issues affecting workers sense of control. In our study, language was not associated with IPM behavior nor was type of salary, which can be an indicator of how pressured a worker may feel on the job (i.e. those workers paid by the pound). Issues of control in this case are perhaps related to the feasibility of engaging in certain IPM behaviors while on the job, such as staying away from a diseased area on the mushroom bed while harvesting. Mushroom harvestors may feel they have little control over staying away from mushroom diseases while picking given the density of the crop. Diseased mushrooms can be in

close proximity to healthy ones, making it difficult for them to work around these areas all while picking as many mushrooms possible. Another issue that control perceptions may be related to is based on the nature of pests and pathogens themselves and farmworkers perceiving a lack of control over their spread within mushroom farms. For example, mushroom flies are extremely small in size and it may seem that even though doors are closed, they still find ways to enter the mushroom farm, causing a low perception of control over the ability to keep them from entering the farms (Keil, 2002).

Knowledge about IPM did not predict IPM behavior. Various studies among farmworkers have found that knowledge or information in itself is not enough to trigger safety behavior. Even though knowledge may inform perceived risk and perceived control, knowledge, information or education alone often will not stimulate a health behavior change (Arcury et al., 2002; Austin et al., 2001; Cabrera & Leckie, 2009),(Halfacre-Hitchcock et al., 2006).

Mushroom farmworkers who work in disease control (as disease spotters) are more likely to engage in IPM behaviors than managers, waterers and harvestors. We did not find any interactions between this group and the other variables measured that would explain this finding. However, a possible explanation may be related to the job description of disease spotters, who are responsible for monitoring the growing rooms scouting for disease spots on the mushroom beds and subsequently covering up the spots with the appropriate control product. Disease prevention is their main duty for which they may be more aware and proactive about mushroom pest and disease preventative (IPM) behaviors. In addition, workers in this position do not get paid by the pound, rather they get paid on a salary basis, more disease on the farm translates into more work for them under the same pay, which may influence a greater concern for mitigating the spread of mushroom disease and mushroom flies (which carry mushroom diseases) through IPM behaviors.

Workers earning an annual income over $30,000 also engaged more frequently in IPM behaviors than those making less than that amount. We did not find any interaction between

farmworker income and other variables that may explain how it influences behavior and whatever statistical explanation for finding goes beyond our data. However, it may be that higher earning workers feel a sense of ownership of their workplace as well as a higher sense of loss if pests and diseases were to affect the productivity of the farm. In this way, income may be acting as a perceived benefit to action influencing IPM behavior.

Conclusions

Our results are consistent with the HBM and have implications for the success of IPM implementation on mushroom farms. IPM interventions should not rely solely on information about mushroom pests and pathogens. Although knowledge and information do begin to inform risk and they should be added to intervention agendas, it is crucial to address possible issues of control keeping farmworkers from engaging in IPM behavior. Further studies are needed in order to dissect pest and pathogen control perceptions among mushroom farmworkers. Even though this study produced valuable information, it is not free from limitations. First, the items we developed to measure perceived risk and perceived control have not been validated and our dependent variable relied on self-reported behavior, a difference between self-reports and actual behavior is possible. Second, we are not able to explain what is affecting farmworker control perceptions regarding mushroom pests and diseases with our data. This is much needed in order to develop IPM interventions that address farmworkers' issues of control surrounding mushroom pests and pathogens which ultimately may lead to a higher implementation of IPM among this population.

Chapter 7

Conclusions

The research presented in the first half (chapters two and three) of this dissertation was aimed at exploring the association between the mushroom sciarid fly *L.ingenua* and the fungal pathogen, *T, aggressivum*, causal agent of mushroom green mold disease. The research presented in chapters four and five was aimed at gathering data about the distribution and activity of the mushroom phorid fly, *M. halterata* outside commercial mushroom farms, as well as the species' population dynamics inside commercial mushroom farms (within mushroom crop cycles). The final research chapter presented had the objectives of describing demographic characteristics of the Hispanic mushroom farmworker population in the Berks and Chester County areas (Pennsylvania), as well as determining how perceptions on mushroom pests and pathogens influence Integrated Pest Management behavior among mushroom farmworkers.

In chapter two I find that *L. ingenua* larvae developing on mushroom compost infested with *T. aggressivum* obtain fitness related benefits in the form of higher percent of adult emergence, faster development from larvae to adult and larger female adult flies, which has been correlated to increased fertility in this species. I conclude that there is likely an association between *L. ingenua* and *T. aggressivum* in mushroom compost. In chapter three I explore the unanswered question of whether *T. aggressivum* benefits from *L. ingenua*. I provide experimental data proving that gravid *L. ingenua* flies mechanically vector (through movement) *T. aggressivum* fungal spores onto petri dishes with nutrient agar, causing new green mold colonies and hence the spread of the pathogen. I also show that female flies vector a larger amount of green mold disease after a pre-oviposition period of 36 hours as opposed to 18 hours. Finally, I show that sciarid larvae also vector green mold through the consumption and excretion of *T. aggressivum* spores. I conclude that *L. ingenua* adults and larvae are able to mechanically vector

green mold disease. In addition, I conclude that association between the two organisms (fly and fungus) is mutual, given that the fly benefits from consuming the fungus and the fungus benefits from the fly in the form of spore dispersal and colonization of new areas through the fly's movement.

Chapter four of this dissertation focuses on behavioral aspects of the mushroom phorid fly, *M. halterata* outside commercial mushroom farms. Particularly I describe the species distribution and abundance among different natural areas commonly found outside farms (spent compost piles, woodrows and turf) as well as the species' flight activity at different times of the day. I conclude that *M. halterata* concentrates over turf areas surrounding mushroom farms, rather than woodrows and spent compost piles, the latter not being an attractive host for the fly given its sterile nature (lacking actively growing mushroom mycelia). I also determine that turf areas are not breeding grounds for *M. halterata*. Finally, I report that *M. halterata* is mostly active between 4 pm and 8 pm, possibly concentrating its' activity before sunset, time when *M. halterata* mating outside mushroom farms was observed.

In chapter five I describe the population dynamics of *M. halterata* within commercial mushroom crops. I discuss that *M. halterata* life cycle from egg to adults within commercial mushroom farming conditions is likely completed in 22-25 days. Overlapping generations as well as two generations within one farming cycle can occur, depending on the time of fly infestation and the length of the farming cycle. I also determine that farming practices such as the type of compost used and organic growing practices are not related to *M. halterata* population densities within mushroom farms. I report that the type of construction of the farm and the density of neighboring mushroom farms, as well as double steaming practices are related to *M. halterata* population densities.

Chapter six is a study of Risk and Control Perceptions around mushroom pests and pathogens among Hispanic mushroom farmworkers. Applying the Health Behavior Model and

with data from 105 surveys, I conclude that high perceptions of risk and control around the spread of mushroom pests and diseases, rather than knowledge about Integrated Pests Management (IPM) predict Integrated pest management behaviors among farmworkers. I conclude that extension efforts to promote IPM on mushroom farms must not be limited to providing information on mushroom pests and pathogens and IPM. Efforts must also be aimed at providing farmworker's with a better sense of control over pest and disease prevention, both at the individual and team level.

Contributions, implications for fly management and future research directions

Previous research on the behavioral and nutritional ecology of *L. ingenua* in the context of mushroom farming has very well established that *L. ingenua*, as a saprophyte, is attracted to and will survive on mushroom compost alone, that is, compost that does not contain actively growing *A. bisporus* mycelia (Cantelo et al., 1982; Kielbasa & Snetsinger, 1981; O'Connor & Keil, 2005). This way, female flies are attracted to mushroom compost even before it has been spawned. Cloonan et al. (2016a; 2016b) , focused on the chemical ecology of *L. ingenua* and advanced this field proving that females mushroom sciarid flies are attracted to microorganisms in unspawned mushroom compost rather than the compost alone. Particularly, they are especially attracted to the fungal causal agent of green mold disease, *T. aggressivum.* The research I present in chapters two and three of this dissertation not only advance Cloonan's break through findings but also contribute to a growing field of research on fungal-insect associations. We have learned that often it is not the host, but the microorganisms present on the host, that are determining insect-host interactions (Stokl et al., 2010; Rohlfs & Churchill, 2011; Becher et al., 2012; Hamby & Becher, 2016; Rohlfs, 2016;) . The work presented in this thesis advances what we know about the particular association between *L. ingenua* and *T. aggressivum* in mushroom compost. The

reported results point to a mutualistic association between the two organisms. The females are ovipositionally attracted to the fungus present on the mushroom compost (Cloonan et al.,2016) which provides the offspring with fitness related benefits (Mazin et al., 2017). In turn, the females physically acquire the spores and mechanically (through movement) spread the spores onto new surfaces, providing a means of spread to the otherwise, sticky and immobile *T. aggressivum* spores.

These findings also expand fly control implications beyond what we knew, which was that fly's cause economic damage to the crop due to their feeding behavior. According to the results presented in this dissertation, fly management becomes even more crucial, given that there is a fitness related association between two organisms, one of which has potentially devastating consequences for a mushroom crop (*T. aggressivum*). The control of mushroom green mold disease must come hand in hand with mushroom sciarid fly control due to the fly's ability not only to vector the disease, but to strive when developing on the fungus. Given that green mold disease is more devastating the earlier it appears in the mushroom crop cycle, special attention should be paid to the early appearance of mushroom sciarid flies as well. The potential synchronicity of these two organisms early in the crop cycle may mean that vectoring of green mold spores will occur at a susceptible time for the crop regarding green mold disease.

The mechanisms in which *T. aggressivum* provides fitness related benefits for *L. ingenua* was not part of the objectives of this dissertation and remain unanswered. Future research is needed in order to determine whether *T. aggressivum* provides nutritional benefits to developing *L. ingenua* larvae that promote a higher survival and a faster development. Another hypothesis that my research raises is that larvae may benefit from the antagonistic effect of *T. aggressivum* towards *A. bisporus* given that *L. ingenua* is able to develop on a fully spawned compost parasitized by *T. aggressivum* as opposed to healthy fully spawned compost. Future research

should explore whether *T. aggressivum* suppresses *A. bisporus* defenses against *L. ingenua* larvae.

Regarding the vectoring of green mold spores by *L. ingenua*, I was not able to characterize the nature of the spore acquisition behavior of gravid *L. ingenua* females. It may be that *L. ingenua* actively collects *T. aggressivum* spores before transporting them to future oviposition sites. This might imply that *L. ingenua* has developed a behavioral adaptation (the active acquisition of spores and the inoculation of its oviposition sites with the fungus) that enhance future offspring fitness. On the other hand, *L. ingenua*, attracted to the fungus, lands on *T. aggressivum* colonies, and that these fungal spores, being of a 'sticky' nature, simply adhere to the flies' exoskeleton and are randomly spread to new areas through the fly's movement.

A limitation of my research is that the vectoring assays were conducted in laboratory settings, with nutrient agar used as a surface to prove the spread of green mold disease by means of the movement of gravid female flies. Future work, perhaps using mushroom compost instead of nutrient agar, is needed to determine what the vectoring dynamics are like in commercial mushroom settings. Potential research questions may be: 1) Does the c.a. 24 hour pre-oviposition period of *L. ingenua* hold true in commercial mushroom settings? And if so, does this period affect the amount of green mold that can be potentially vectored by the fly? 2) How does *T. aggressivum* pathogenicity occur after it is vectored by *L. ingenua* onto mushroom compost? For example, in what amount of time do the vectored spores develop into a disease and how many spores are needed to be vectored by the fly to cause a significant amount of green mold disease? 3) Are *L. ingenua* densities within commercial mushroom growing rooms correlated to the amount of green mold colonies in those same rooms?

Previous mushroom phorid fly research conducted outside commercial mushroom farms was conducted in Europe, with most of the work done over 30 years ago (Hussey, 1965). Until now, no information on behavioral aspects of *M. halterata* outside mushroom farms in North

America was available in the scientific literature. The work on the activity and distribution of *M. halterata* outside commercial mushroom farms in Chester County PA presented in this thesis is the first of its kind. Previously, observations on *M. halterata* outside commercial mushroom farms had been anecdotal. Mushroom farm owners and farmworkers in PA had reported what appeared to be mushroom phorid flies swarming outside their farms. In addition, anecdotal reports also described how flies were seen trying to leave the growing rooms when the crop was in a production phase. The data presented in chapter four confirms that mushroom phorid flies are in fact active outside commercial mushroom farms. Outside activity includes mating, which we were able to confirm through visual observations and trapping of adults in copula. An important implication of this finding is that *M. halterata* can spend a part of its lifetime outside the mushroom farm. This behavior can be targeted through trapping or through mating disruption outside mushroom farms. Some questions that remain unanswered regarding this behavior are: 1) Whether the flies exiting the farm is a response to an overpopulation of flies in the production phase of the crop or if it is a response to an attraction to natural light, since at this time the farm doors are opened. 2) Although unlikely, is mating exclusive to the outdoors or does it also occur inside the growing rooms? 3) Are the flies caught outside growing rooms part of a different (feral) population of *M. halterata* or are they from the same population from within the growing rooms?

The data presented on the daily flight activity of *M. halterata* will render useful for the timing of future control methods aimed flies outside growing rooms. Growers can schedule growing practices that involve opening farm doors around times that are reported to be of high fly activity outside the farms (from 4 pm to after sunset), in order to reduce the risk of invading flies into the growing rooms. A limitation of this study was that sampling was not conducted on an hourly basis, for which certain temperature relationships within sampling intervals were not elucidated. For example, in August of 2016, sampling during the evening occurred from 4 pm to

midnight. During this interval, we were not able to determine exactly when the observed cessation of flight activity occurred and whether it was due to lower temperatures, a lack of light after sunset or both. It would be very valuable if future research involved hourly fly sampling with the recording of on-site hourly temperatures. This would help establish a more precise relationship between *M. halterata* flight activity and outside temperature.

We were able to confirm that pasteurized, spent mushroom compost is not an attractive host for *M. halterata*. Our results reinforce the notion that growers should always 'steam-off' the mushroom compost before it is removed from the room, not only for sanitation and disease control purposes but for fly control as well. Sometimes spent compost is left on the premises for some days before its removal, sterilizing the compost will assure that mushroom phorid flies will not lay eggs in it and cause re-infestations. Often, spent mushroom compost is used as a fertilizer in agricultural fields and gardens (Phan & Sabaratnam, 2012). Based on our findings, sterilized spent mushroom compost should not be a case for concern regarding future mushroom phorid fly infestations in other areas where the substrate is utilized.

Mushroom phorid fly research within commercial mushroom farms in Pennsylvania had not been published since the 1980's (Rinker, 1982). The research on mushroom phorid fly population dynamics presented in this dissertation was similar to other studies of its kind yet includes new findings and points to future directions for *M. halterata* research on commercial mushroom farms. Our data showed trends regarding phorid fly emergence within the mushroom growing cycle, which are in accordance with *M. halterata* previous temperature-related life cycle reports (Hussey, 1960; Richardson & Hesling, 1978; Barzegar et al., 2016). The data suggests that phorid fly lifecycle within mushroom farming conditions can take 21-25 days depending on compost temperatures. Emergence of the first generation of flies will depend on which days of spawn run fly invasion ocurred. One limitation of our study is that we did not measure compost temperatures at the time of fly sampling. Rather, our estimation of fly emergence relied on

temperatures reported by growers. The temperature gradient within the mushroom growing substrate (compost) in commercial mushroom farms fluctuates depending on the depth of the compost as well as the crop stage. The development of *M. halterata* is extremely temperature dependent (Barzegar et al., 2016), there for, the effect of fluctuating mushroom compost temperatures on phorid fly development should be studied in order to build more precise *M. halterata* lifetables that apply to shifting mushroom farming conditions and that can predict fly emergence. Nonetheless, our data does suggest that fly control should be emphasized from the first day of spawn run until at least the last day of casing. One generation should be expected and in occasions where fly invasion occurs constantly during spawn run and case hold, an overlap of generations may also occur. In addition, when the crop is extended to third break, two generations of *M. halterata* within the same crop can also occur.

Similar to Rinker (1982) and to Navarro (2015) we found a trend in the population fluctuation of *M. halterata* at the time of casehold; a large peak in fly counts on the monitors was recorded between day 14 and day 17 of the growing cycle. Further experiments are needed in order to determine whether these peaks are representative of invading flies when mushroom farms doors are opened at casing or if they represent those flies that are on the compost at the time of casing and have been physically disturbed (and have flown up into the monitor) due to the casing operation. Elucidating the cause of these peaks will have practical implications regarding fly management. If flies invade the rooms in large quantities at the moment of casing, control measures can be taken the day of casehold or shortly afterwards in order to target ovipositing female flies and mitigate the emergence of a future generation. In the case that the increase of fly counts on the monitors corresponds to disturbed flies, an alarming possibility arises: that the fly numbers recorded during spawn run (before casing) are not representative of the amount of flies present on the mushroom bed at this stage. Often the fly numbers causing the peaks recorded on the day of casing were three fold the number recorded during spawn run. It may be that these

numbers are representative and drastically underestimate the amount of flies present in the compost. Finally, if mushroom phorid flies do not invade the growing rooms the day of casing, the application of pesticides the day of or after casing (a common practice in the industry) is unnecessary.

This dissertation presents the first study on farming practices related to *M .halterata* density on commercial mushroom farms using a regression analysis. One limitation of this analysis is that the data was not obtained from a randomized controlled study, rather, it was observational and the regression lacks predictive power. Randomizing a study in commercial mushroom farms is very difficult because practices cannot be altered on commercial farms without affecting their productivity. However, the variability between farming practices in Chester County PA is minimal, which renders our results likely to be applicable to a large sample of farms in the area. The practices we studied (compost, steaming empty and organic as opposed to non-organic growing) as well as the factors related to farm construction (Dutch farm and conventional farms) and fly pressure (whether a farm is densely surrounded by other farms or not) are applicable to any mushroom farm in Chester County.

The results regarding growers observations about 'fly pressure' (fly pressure refers to the likelihood of having more mushroom fly problems if a farm is in a farm-dense area) presented in this thesis confirm these observations. The number of neighboring farms as well as their proximity had a positive effect on mushroom phorid fly densities on farms, which points to the need for area-wide phorid fly management efforts which must be coordinated between neighbors. Regarding the compost, growers perhaps should not be concerned about the type of compost that they fill as a factor influencing fly populations on their farms since we did not find any significant difference in fly numbers between the types of compost utilized (phase I or phase II). One finding that we could not explain was that those farms employing double-steaming practices (see introduction section in chapter 5) had higher fly numbers than those that did not. Farms in

Chester County that do not double steam growing rooms typically fill phase I mushroom compost. We did not find any interaction between type of compost and double steaming practices. Phase one mushroom compost contains a high level of ammonia (Beyer, 2003; Pecchia & Beyer, 2013), it may be that the ammonia in the room kills any flies that remain in the empty rooms before they are filled (we have anecdotal data confirming that flies remain in the rooms even though they are empty). It may also be that the pasteurization of the phase I compost within the growing rooms kills any lingering flies within those same rooms as well. Future research should focus on the possible effects of ammonia on mushroom flies as well as *M. halterata* activity in growing rooms between crops.

Future studies should also focus on *M. halterata* pesticide resistance, given that our study did not find any differences in fly numbers between organic and non-organic farms. This may mean that chemical control methods are ineffective and that their over use is possibly harnessing a resistant *M. halterata* population, which also calls for the development of alternative fly control methods.

Finally, the data presented in this thesis regarding mushroom farmworker (demographic) characteristics as well as risk and control perceptions as well as levels of knowledge around mushroom pests and pathogens is the first of its kind. Hispanic mushroom farmworkers in Berks and Chester County PA are crucial to the commercial success of mushroom farms. They perform important tasks on a day to day basis such as harvesting, watering, controlling mushroom diseases and even managing crops from start to finish. Embedded in these tasks are mushroom integrated pest management programs that rely on the implementation of techniques that prevent the establishment and spread of mushroom diseases. In this dissertation we use for the first time the health behavior model in the study of IPM behavior among mushroom farmworkers. Our findings contribute to existent studies of risk mitigating behavior among Hispanic mushroom farmworkers that prove that risk and ultimately control perceptions affect behavior more so than knowledge

and education do (Vaughan, 1993;Grieshop et al., 1996; Austin et al., 2001;Arcury et al., 2002; Cabrera & Leckie, 2009) . One limitation of our study is that we did not go further into exploring what belief systems and work environment issues might possibly be affecting farmworkers perceptions of risk and control over mushroom pests and diseases. This could be done in future research through qualitative studies. In addition, future studies may want to implement researcher observations on IPM behavior, rather than rely on self-reported IPM behavior as the study presented here did. Differences between self-reported and actual behavior are always possible.

However, our study does pose important implications for future IPM intervention efforts among mushroom farmworkers. First, efforts must not be limited to handing out information or on educating about mushroom IPM. Second, risk perceptions are related to how susceptible and person perceives themselves to be of a risk as well as the consequences of the risk (Rosenstock et al., 1988), perhaps IPM intervention efforts should include detailed explanations of the threat that pests and diseases impose to mushroom crops as well as the consequences. Lastly, control perceptions reflect the degree to which a person feels he/she is able to perform a behavior as well the degree to which they are able to influence a risk outcome (Janz & Becker , 1984). This implies that intervention efforts should include practical demonstrations on how personal IPM behaviors can in fact affect pest and disease outcomes on farms.

Part of the theoretical framework presented in the IPM behavior study is that IPM is a form of reducing farmworker pesticide exposure through the reduced use of pesticides (result of an effective IPM program). The extent to which IPM reduces the need and the application for pesticides in mushroom farming as well farmworker exposure rates has not been studied or reported in the literature. This would render useful not only for the advocacy of IPM on mushroom farms in general, but for pushing IPM further into public policy as well as an issue of public health and farmworker justice.

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Appendix

Survey: Farmworker mushroom pest and pathogen risk perceptions

Thank you for agreeing to answer this survey. I remind your answers are confidential. You can always refuse to answer any questions and you can stop the survey at any time if you wish. You may also ask questions at any time. Let's begin.

1. Do you know what integrated pest management is?
   1 yes (GO TO THE NEXT QUESTION 2)

   2 no (GO TO QUESTION 3)

2. Can you briefly state what it is?

3. Do you agree with the following statements?

Mushroom diseases can stick to working tools.	1 Yes	2 No	3 I don't know	4 Refuse
Mushroom diseases can stick to clothing	1 Yes	2 No	3 I don't know	4 Refuse
Mushroom diseases can spread from one growing room to another trough working tools.	1 Yes	2 No	3 I don't know	4 Refuse
Mushroom diseases can spread from one growing room to another through clothing.	1 Yes	2 No	3 I don't know	4 Refuse
Mushroom flies can spread mushroom diseases.	1 Yes	2 No	3 I don't know	4 Refuse
Mushroom flies look for newly spawned compost to lay eggs in.	1 Yes	2 No	3 I don't know	4 Refuse
In order for the mushroom disease to be present visible signs must exist.	1 Yes	2 No	3 I don't know	4 Refuse
The best tool for managing mushroom diseases is always prevention of the disease.	1 Yes	2 No	3 I don't know	4 Refuse

4. Have you ever received any type of information on how to manage mushroom flies and mushroom diseases?

1 Yes (GO TO QUESTION 5)
0 No (GO TO QUESTION 9)

5. How long ago was this information given to you?

1 Within the past 0 to 6 months
2 Within the past 6 months to 2 years
3 Within the past 2 to 4 years
4 Over four years ago

6. How was this information on mushroom flies and diseases given to you?

1 My supervisor told me about mushroom flies and diseases
2 I assisted an educational oral presentation on mushroom flies and diseases
3 I was given a pamphlet/fact sheet
99 No response

7. In what language was this information given to you?

1 English
2 Spanish
99 No response

8. How well did you understand the information about mushroom flies and diseases that was given to you?

1 not at all
2 somewhat
3 a little
4 well
99 No response

9. How would you prefer that information on mushroom flies and diseases be given to you in the future?

1 oral presentation/educational session

2 pamphlet

3 direct communication from my supervisor

99 No response

Great, thank you. Now I will ask you about your thoughts on mushroom pests and pathogens. I will read some common situations that happen on mushroom farms, I will give you answer choices, choose which one best describes your thoughts.

10. The doors to a growing room are not closed and flies enter the growing room.
    - 1 This is a problem that I don't worry about
    - 2 This is a problem I worry very little about
    - 3 This is a problem I worry moderately about
    - 4 This is a problem I worry a great deal about
    - 77 Not sure
    - 99 No response

11. A mushroom diseases like green mold or dry bubble spreads from one growing room to another or from one part of the farm to another?
    - 1 This is a problem that I don't worry about
    - 2 This is a problem I worry very little about
    - 3 This is a problem I worry moderately about
    - 4 This is a problem I worry a great deal about
    - 77 Not sure
    - 99 No response

12. A mushroom disease like green mold or dry bubble causes yield losses to a mushroom crop?
    - 1 This is a problem that I don't worry about
    - 2 This is a problem I worry very little about
    - 3 This is a problem I worry moderately about
    - 4 This is a problem I worry a great deal about
    - 77 Not sure
    - 99 No response

13. Do you worry about how to control mushroom flies?
    - 1 I do not worry about this
    - 2 I worry very little about this
    - 3 I worry moderately about this
    - 4 I worry a great deal about this
    - 77 Not sure
    - 99 No response

14. Do you worry about how to control mushroom diseases (like green mold or dry bubble)?

1 I do not worry about this
2 I worry very little about this
3 I worry moderately about this
4 I worry a great deal about this
77 Not sure
99 No response

15. How much control do you believe you have over keeping diseases (i.e. green mold, dry bubble) from spreading on the mushroom farm (i.e. one area of the bed to another or one growing room to another)?

1 No control
2 Small amount of control
3 Moderate amount of control
4 A great deal of control
77 Not sure
99 No response

16. How much control do you believe you have over keeping flies from spreading from one mushroom house to another?

1 No control
2 Small amount of control
3 Moderate amount of control
4 A great deal of control
77 Not sure
99 No response

17. How much control do you believe you have of avoiding areas where you can see disease signs?

1 No control
2 Small amount of control
3 Moderate amount of control
4 A great deal of control
77 Not sure
99 No response

18. How much control do you believe have over wearing freshly washed clothes every day?

1 No control
2 Small amount of control
3 Moderate amount of control
4 A great deal of control
77 Not sure
99 No response

19. Is disinfectant material provided on the farm every day for you to wash your work tools?

1 Yes (GO TO 20)
2 No (GO TO 23)
3 I don't know
99 No response

20. How far away is the disinfectant material for tools?

1 Less than 1 minute
2 Between 1 and 5 minutes
3 More than 5 minutes
77 Don't know
99 No response

21. Is hot water and soap provided on the farm every day for you to wash your hands?

1 Yes, both (GO TO 16)
2 Yes, hot water
3 Yes, soap only
4 No, neither (GO TO 17)
5 Yes, cold water only
99 No repsonse

22. How far away is the water for washing hands?

1 Less than 1 minute
2 Between 1 and 5 minutes
3 More than 5 minutes
77 Don't know
99 No response

23. Where do you wash your work clothing?

1 Home
2 Laundromat
3 Other
77 Don't know
99 No response

Thank you for your responses. The next questions will be about things you do on the farm. Remember that it doesn't matter how much you do them it matters that you give an honest response. Let's begin.

24. Which best describes the work you do on this farm? You may choose more than one option.

1 picker (GO TO QUESTION 25).
2 irrigation staff (GO TO QUESTION 26).
3 disease spotter (GO TO QUESTION 26).
4 crop manager (GO TO QUESTION 26).
77 Don't Know
99 Refuse

25. As a picker: How much control do you have over washing your tools at the beginning of your work day?

1 No control
2 Small amount of control
3 Moderate amount of control
4 A great deal of control
77 Not sure
99 No response

26. In the past week how many times have you done this?

	Crop manager	Irrigation	Disease Spotter	Harvestor
a.Wear freshly washed clothes to work	1 5-7 days 2 3-4 days 3 1-2 days 4 0 days	1 5-7 days 2 3-4 days 3 1-2 days 4 0 days	1 5-7 days 2 3-4 days 3 1-2 days 4 0 days	1 5-7 days 2 3-4 days 3 1-2 days 4 0 days
b.Close doors when you enter a room	1 5-7 days 2 3-4 days 3 1-2 days 4 0 days	1 5-7 days 2 3-4 days 3 1-2 days 4 0 days	1 5-7 days 2 3-4 days 3 1-2 days 4 0 days	1 5-7 days 2 3-4 days 3 1-2 days 4 0 days
c.Report a disease to your supervisor if you see it on the mushroom bed	1 5-7 days 2 3-4 days 3 1-2 days 4 0 days	1 5-7 days 2 3-4 days 3 1-2 days 4 0 days		1 5-7 days 2 3-4 days 3 1-2 days 4 0 days
d.Stay away from a diseased area on the mushroom bed while working		1 5-7 days 2 3-4 days 3 1-2 days 4 0 days	1 5-7 days 2 3-4 days 3 1-2 days 4 0 days	1 5-7 days 2 3-4 days 3 1-2 days 4 0 days
e.Cover a hole in the wall/door/roof if you see it	1 5-7 days 2 3-4 days 3 1-2 days 4 0 days			
f.Wash your tools before starting your workday				1 5-7 days (GO TO 27) 2 3-4 days (GO TO 27) 3 1-2 days (GO TO 27) 4 0 days

27. With what material do you wash your tools?

1 Water
2 Water and soap
3 Bleach or Hydrogen peroxide
77 Not sure

99 Refuse

28. I'm going to read you some scenarios. Please tell me whether you thik these scenarios affects you personally, affect the mushroom, affects both yourself and the farm or neither.

	Health	How?
a.Closing the door when entering a room affects:	1 You 2 Mushroom 3 Both 4 Neither	1.Production 2.Income 3. Health 4.Disease 5.Happy mushroom 6.Happy boss 7.Less flies 8. Less pesticides 9.Prevention 10. Comfort
b. Staying away from mushroom diseases on the bed affects:	1 You 2 The farm 3 Both 4 Neither	1.Production 2.Income 3. Health 4.Disease 5.Happy mushroom 6.Happy boss 7.Less flies 8. Less pesticides 9.Prevention 10. Comfort
c. Washing work tools every day affects:	1 You 2 Both 3 Both 4 Neither	1.Production 2.Income 3. Health 4.Disease 5.Happy mushroom 6.Happy boss 7.Less flies 8. Less pesticides 9.Prevention 10. Comfort
d. Wearing clean clothes to work affects:	1 You 2 The farm 3 Both 4 Neither	1.Production 2.Income 3. Health 4.Disease 5.Happy mushroom 6.Happy boss 7.Less flies

		8. Less pesticides 9.Prevention 10. Comfort

We are almost done!
The following questions ask for information about yourself that will help us understand your answers a little better. Here we go.

(Demographic questions)

29. What is your age?

_ _ Code age in years
77 Don‘t know / Not sure
99 Refused

30. What is your gender?

31. How are you paid?

1 by the hour
2 by the pound
3 combination hour and pound
4 salary
99 No response

32. What was your total personal income last year in U.S. Dollars (Choose One)?

1. I did not work at all in the past year
2. Less than 500
3. 500 to 999
4. 1,000 to 2,499
5. 2,500 to 4,999
6. 5,000 to 7,499
7. 7,500 to 9,999
8. 10,000 to 12,499
9. 12,500 to 14,999
10. 15,000 to 17,499
11. 17,500 to 19,999
12. 20,000 to 22,499
13. 22,500 to 24,999

14. 25,000 to 27,499
15. 27,500 to 29,999
16. 30,000 to 32,499
17. 32,500 to 34,999
18. 35,000 to 37,499
19. 37,500 to 39,999
20. Over 40,000
21. Don't remember (Don't know)

33. Are you…?

1	Married/Living with someone
2	Divorced
3	Widowed
4	Separated
5	Single
	Or
6	A member of an unmarried couple
	Do not read:
99	Refused

34. Where were you born (Country, State)

1	United States
2	Mexico
66	Other
77	Don’t know/Not sure
99	No response

35. What is the highest grade in school you completed?

Read only if necessary:

1	Never attended school or only attended kindergarten
2	Grades 1 through 8 (Elementary)
3	Grades 9 through 11 (Some high school)
4	Grade 12 or GED (High school graduate)
5	College 1 year to 3 years (Some college or technical school)
6	College 4 years or more (College graduate)
	Do not read:
99	Refused

36. How many years have you been working in agriculture?

_ _ Code age in years

77 Don‘t know / Not sure

99 Refused

37. How many years have you been working on mushroom farms?

_ _ Code age in years

77 Don‘t know / Not sure

99 Refused

38. How many years have you been working on this farm?

_ _ Code age in years

77 Don‘t know / Not sure

99 Refused

39. How well do you speak English?

1 not at all
2 somewhat
3 a little
4 well

40. How well does your supervisor speak Spanish?

1 Not at all
2 Somewhat
3 A little
4 Well

VITA

Maria Mazin

Maria Mazin grew up in California and Mexico City. She obtained a bachelor's degree in Agricultural Sciences specializing in plant protection with honors from Universidad Veracruzana in Mexico. Her bachelor's thesis reported the host switch of the codling moth, *Cydia pomonella from apple orchards to Magnolia* spp. trees in a natural reserve in Veracruz, Mexico. Since university, she developed a passion and interest for applied entomology and Integrated Pest Management, especially in agricultural settings. During and after her bachelor's degree, Maria worked with the agricultural extension office of Universidad Veracruzana, giving IPM workshops to indigenous farmers in the region.

As part of her PhD degree in Entomology and International Agriculture and Development, Maria studied mushroom flies *Megaselia halterata* and *Lycoriella ingenua* in both laboratory and commercial mushroom field settings. Her research focused on the association between *L .ingenua* and green mold disease and on the ecology and population dynamics of *M. halterata* in and around commercial mushroom farms. Bridging the field of Entomology and Agricultural development together, she worked with Hispanic mushroom farm workers, studying their perceptions on IPM and mushroom pests and diseases in order to include farmworkers in the IPM conversation and develop culturally appropriate and effective IPM educational programs on mushroom farms.