Evaluation of *Beauveria bassiana* applications against adult house fly, *Musca domestica*, in commercial caged-layer poultry facilities in New York state

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**Abstract**

Applications of a commercially produced *Beauveria bassiana* product, balEnce, were compared with pyrethrin treatments for the control of adult house flies in New York high-rise, caged-layer poultry facilities. An integrated fly management program, which included the release of house fly pupal hymenopteran parasitoids, was used at all facilities. Adult house fly populations were lower in *B. bassiana*-treated facilities during the spray and post-spray periods, as recorded on spot cards. Concurrently, the numbers of house fly larvae recovered in *B. bassiana*-treated facilities were less than one-half that of the pyrethrin-treated facilities. House fly pupal parasitism levels were low, but similar under both treatment regimes. The numbers of adult and larval *Carcinops pumilio*, a predatory beetle, recovered from *B. bassiana*-treated facilities were 43 and 66% greater than from the pyrethrin-treated facilities, respectively.

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1. Introduction

House fly, *Musca domestica* L., is a major pest of poultry production (Axtell, 1999). The development of pesticide resistance by the house fly (Scott et al., 2000) has increased the willingness of producers to seek alternative methods for fly management. The industry has readily adopted integrated approaches appropriate to their production facilities (Axtell, 1999; Kaufman et al., 2002). The use of fungal pathogens is an area of poultry fly management that has garnered attention, but until recently, there has been no facility-wide success.

The fungal pathogen, *Beauveria bassiana* (Balsamo) Vuillemin, has been recorded from hundreds of insect species (Fargues and Remaudiere, 1977). Steinkraus et al. (1990) first reported the natural occurrence of *B. bassiana* in the house fly. Additionally, numerous studies are available documenting the attempted use of fungal pathogens against house flies in laboratory and field experimentation including *Entomophthora muscae* (Cohn) Fresenius (Geden et al., 1993; Mullens et al., 1987; Watson and Petersen, 1993) and *B. bassiana* (Watson et al., 1995, 1996). Additional pests of poultry also have been targeted with the use of *B. bassiana* against the lesser mealworm, *Alphitobius diaperinus* (Panzer) and the hide beetle, *Dermestes maculates* DeGeer (Crawford et al., 1998; Geden et al., 1998; Geden and Steinkraus, 2003).
Attempts have been made in cropping systems with commercialization of *B. bassiana* against several pests. Results of applications against the Colorado potato beetle have been highly variable and foliar applications have not provided commercially acceptable control (Hajek et al., 1987; Wraight and Ramos, 2002). Encouraging results and commercial products based on *B. bassiana* are available targeting whiteflies, aphids, thrips, and mealybugs in greenhouses and nurseries (Faria and Wraight, 2001).

Caged-layer poultry facilities are rich in arthropod diversity (Axtell, 1999; Hinton and Moon, 2003; Kaufman et al., 2002) and producers are most willing to utilize indigenous and commercially purchased natural enemies for fly control. The effect of *B. bassiana* against the beneficial organisms common in poultry facilities has been minimally studied (Geden et al., 1995), but an understanding of the impact of this potential management tool is critical if it is to be effectively utilized by producers for fly control.

Until the current study, there have been no published studies documenting successful applications of commercially produced *B. bassiana* formulations targeting adult house flies in caged-layer poultry facilities. We examined the efficacy of a commercially available formulation of *B. bassiana* against naturally occurring house fly populations following manure removal in high-rise, caged-layer poultry facilities in New York. Additionally, we examined the dynamics of indigenous and introduced beneficial arthropod populations in both *B. bassiana*-treated and pyrethrin-treated facilities.

### 2. Materials and Methods

#### 2.1. Facilities and Treatments

The study was conducted in four high-rise, caged-layer poultry facilities on farms in Onondaga and Wayne Counties, New York, USA. No residual pesticides were applied in the facilities within 6 weeks of the study initiation. Manure was completely removed from facilities at Farm A on 18 April, 2003, and at Farm B on 21 April, 2003, immediately prior to initiating the study. After preset fly threshold levels (defined below) were reached, one of the facilities at each farm received *B. bassiana* applications on a schedule as described below. The second facility on each farm served as a *B. bassiana*-untreated control and subsequently received pyrethrin applications as needed.

The *B. bassiana* product under development, balEnce, was obtained from Jabb of the Carolinas (Pine Level, NC). balEnce is a proprietary formulation of 10 g of $5 \times 10^{11}$ conidia of *B. bassiana*, originally isolated from adult house flies, suspended in 15 ml oil mixed with an emulsion agent. Applications were made using Solo 450 (Solo, Newport News, VA) backpack mistblowers calibrated to allow a coarse fog of 40 μm or larger to be applied as the applicator walked the length and back of the pit. The fog was directed at the beams supporting the bird level of the facility and slightly below beam level and the entire pit airspace was covered. The amount of water per application was determined by the size of the building and the speed at which the applicator walked, approximately 13 liters/3000 m². The product was delivered at a rate of application equal to $2 \times 10^8$ *B. bassiana* conidia/m²/week (2 bottles/week), applied over 4 weeks. Producers did not utilize residual premise or space insecticide applications in the facilities where *B. bassiana* was applied; however, methomyl-based fly baits were utilized on the bird level in all four of the facilities. At Farm A $\approx 10.5$ kg fly bait/week/facility was used between study weeks 4 and 7 and at Farm B $\approx 0.5$ kg fly bait/week/facility was used between study weeks 3 and 5.

Due to high house fly populations and fly complaint challenges with nearby neighbors, both producers utilized pyrethrin-based insecticides in the non-*B. bassiana* facilities to reduce adult fly numbers. Farm A used Whitmore HydroPy-300 (3% pyrethrin, 6% piperonyl butoxide) in a mixture that consisted of 1.9 liters of concentrate into 11.4 liters of water and was applied using a separate SOLO 450 mistblower as described above. Farm B utilized 4 liters of Prentox Pyronyl Oil Concentrate (3% pyrethrin, 6% PBO) in a thermal fogger with no additional water for each application. It became necessary to apply several applications as described later.

Treatments with *B. bassiana* on Farm A began on 07 May, 2003, and continued through 12 June, 2003, with 22 total applications using 12 bottles of product. The first two applications were made with a full bottle of product ($1 \times 10^8$ conidia/m²). The remaining applications were at the rate of one-half bottle per application ($5 \times 10^7$ conidia/m²) in order to increase the probability of fly-conidia contact, while not increasing the cost of the product used. In all cases, the balEnce product was diluted into water for delivery through the mistblower, as described earlier. On Farm B, *B. bassiana* applications began on 09 May, 2003, and ended on 15 June, 2003, with a total of 22 applications and 11 bottles of *B. bassiana* product, all at the one-half bottle per application rate. Pyrethrin treatments at Farm A began on 19 May, 2003, and were completed on 12 June, 2003, totaling nine in number. The non-*B. bassiana*-treated facility on Farm B was treated with pyrethrins on 11 dates beginning on 10 May, 2003, and ending on 11 June, 2003. All pyrethrins treatments were made at the label rate of 1%.

The house fly parasitoids, *Muscidifurax raptor* Girault and Sanders and *M. raptorellus* Kogan and Legner (Hymenoptera: Pteromalidae), were purchased from a commercial insectary (IPM Laboratories, Locke, NY) and released into each of the four facilities by producers.
in a 50:50 adult parasitoid ratio during study weeks 2–8. The specific levels of weekly releases were timed to correspond with expected house fly outbreaks and thus differed week to week. An average of 3.5 house fly parasitoids/bird were released weekly into each of the four facilities with greatest releases (6 parasitoids/bird) occurring during weeks 3–5.

2.2. Arthropod sampling

As B. bassiana applications were but one component in the IPM program, it was critical to have an understanding of the arthropod population dynamics in these facilities. Therefore, monitoring of facilities included the sampling of adult and larval house flies, house fly parasitoids, and predators in all facilities.

Spot card and moving sticky ribbon counts were used to assess the weekly relative abundance of adult house flies present in the manure pit. Spot cards, 10 per manure pit, consisted of 76 × 127mm white file cards placed on the manure pit support beams, 1.5m above the pit floor (Axtell, 1970). Cards were replaced weekly and the total number of fly spots on one-half of the card was counted using a uniform grid. Additionally, in each facility a walking sticky ribbon count was performed weekly (Turner and Ruszler, 1989). Sticky ribbon adult house fly monitoring was achieved by walking down one aisle and back another of the manure pit holding a 45cm sticky ribbon (Victor, Woodstream, Lititz, PA) vertically with two hands directly in front of the researcher at arms length from the body. The number of flies captured was determined.

House fly parasitism rates were monitored weekly using the sentinel house fly pupal method of Rutz and Axtell (1980). Ten sentinel bags (5.5 squares/cm nylon mesh), each containing 30 live house fly puparia, were placed weekly on the surface, near the base of the manure pile. After 7 days the puparia from each bag were retrieved and held in the laboratory for 8 weeks to allow for fly and parasitoid emergence. Parasitoids were counted, sexed, and identified to species.

House fly predators, darkling beetles, and house fly larvae were monitored using a bulb planter (400 ml) to collect two manure cores from each of 10 sites/facility/week. Arthropods were extracted in Berlese funnels and enumerated (Geden and Stoffolano, 1988; Kaufman et al., 2002). House fly breeding potential was determined weekly at 10 sites by measuring the number of centimeters from the manure cone peak to one edge of the manure pile. Within that zone, the number of centimeters that contained first and second stage house fly larvae was determined. This measurement represented active breeding and was divided by the peak to outer edge measurement to determine the percentage breeding. Third stage larvae are more transient and move to drier areas lower on the manure pile seeking pupation sites making their presence an unreliable indicator of fly breeding.

The B. bassiana treatment initiation threshold incorporated both spot card and sticky ribbon adult house fly sampling methods described previously. When either the average number of spots on cards from the B. bassiana-treated facility exceeded 100 spots per card or 50 flies on a sticky ribbon, the producer initiated B. bassiana applications. Two applications per week were made for a minimum of 4 weeks. Following the 4 weeks of application, spot card and sticky ribbon counts were again examined. If the average number of spots was above 200 per card or 100 or more flies were counted on a sticky ribbon, additional B. bassiana sprays were to be made with a maximum of 2 additional weeks of adult fly control applications. However, if the measure was under 200 spots or 100 flies, no further sprays were to be applied. In following the study protocol, applications of B. bassiana were indicated and were, therefore, made for an additional 2 weeks past the initial 4-week application period.

2.3. Statistical analysis

In this study, two farms, each operating two similar facilities were chosen in order to minimize variability between management systems. Although adult house fly outbreaks typically occur within 6 weeks of new manure accumulation, the exact timing is dependent upon many environmental and management factors. Therefore, each facility was treated independently of the others with respect to timing of B. bassiana and pyrethrin applications. However, data used in the analysis were specifically allocated to the proper treatment period (pre-spray, spray or post-spray).

Spot card, sticky ribbon, breeding potential, and manure core arthropod data were log transformed and analyzed separately by treatment period using a three-way analysis of variance, ANOVA, with study week, farm, and treatment in the model (Proc GLM; SAS Institute, 1996). The average percentage of sentinel house fly pupae killed (total parasitism) and the percentage successful parasitism (those house fly puparia producing adult parasitoids) were determined for each facility and treatment period. The percentage of fly puparia successfully parasitized was calculated by dividing the number of puparia with emergence holes by the total number of puparia retrieved. Percentage of pupae killed was corrected for control mortality using the method of Abbott (1925). Percentages were arcsine transformed for statistical analysis. The percentage of house fly puparia successfully parasitized and the percentage total parasitism were analyzed using a multi-factorial ANOVA model (Proc GLM; SAS Institute, 1996) to detect differences between B. bassiana-treated facilities and untreated facilities within treatment periods. All data presented in tables are displayed as untransformed means.
3. Results and discussion

We attempted to utilize two tools to measure adult house fly populations in the facilities: spot cards and sticky ribbons. However, due to facility design differences in the position of posts relative to manure pit walkways, the sticky ribbon data became skewed. In one facility, building support posts were positioned in the walkways between manure rows. These walkways were also the only avenue for movement through the facility and were therefore utilized in adult fly sampling with the sticky ribbon. When walking the sticky ribbon, adult house flies that were resting on the support posts flew from the posts and were captured on the sticky ribbon, greatly increasing their numbers on the ribbons. This was an unanticipated problem and did not become apparent until the adult fly numbers had become quite high. Therefore, although a useful tool in many situations, sticky ribbon data were not an appropriate measure of adult house fly populations in this study and were therefore not included in the analysis.

When spot card data were examined within study time period (pre-treatment, spray period, and post-treatment), an overall picture of B. bassiana effectiveness emerges (Table 1). Spot card data documented that significantly more flies were present in the pyrethrin-treated facilities during the spray ($F = 12.78$; $df = 1, 12$; $P \leq 0.004$) and post-spray ($F = 7.48$; $df = 1, 5$; $P \leq 0.041$) period, indicating that the B. bassiana treatments successfully reduced fly abundance as compared to a standard fly management program. We found that fly levels were 21 and 43% lower in B. bassiana-treated facilities than in pyrethrin-treated facilities during the treatment and post-treatment period, respectively.

The large discrepancy in the amount of fly bait utilized is not believed to have had a major impact on the results in this study for two reasons. First, baits were only applied in the bird level of the facility, largely as an attempt by producers to reduce the prevalence of adult flies in the egg processing area. Second, due to treatment pairing at both farms and the duplicative fly bait application rates in facilities at each farm, a similar impact on the fly population in each facility is likely to have existed.

3.1. Non-target arthropods

It was thought that the use of B. bassiana would enhance biological control and ultimately house fly management by conserving populations of beneficial arthropods in the poultry manure. However, successful house fly parasitism was quite low in this study and resembled parasitism levels reported from facilities where commercial parasitoid releases were not conducted (Table 2) (Henderson and Rutz, 1991; Merchant et al., 1987; Rutz and Axtell, 1979; Rutz and Sceles, 1989). The percentage of total parasitism was considerably higher than successful parasitism; however, levels were lower than that observed in previous studies and were not significant ($F = 0.44$; $df = 1, 6$; $P \leq 0.531$) (Kaufman et al., 2001a, 2002). Significant differences in successful parasitism and total parasitism were not observed between the two treatment regimes. This suggests that treatment with B. bassiana was, at a minimum, no worse than that observed in systems using non-residual premise material.
Successful parasitism and total parasitism of sentinel house fly pupae from four New York caged-layer poultry facilities that received either *B. bassiana* or pyrethrin applications for house fly control

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pre-spray</th>
<th>Spray</th>
<th>Post-spray</th>
</tr>
</thead>
<tbody>
<tr>
<td>Successful parasitism&lt;sup&gt;b&lt;/sup&gt;</td>
<td><em>B. bassiana</em></td>
<td>6.3 (4.8) NS</td>
<td>14.5 (4.5) NS</td>
</tr>
<tr>
<td></td>
<td>Pyrethrin</td>
<td>2.3 (2.2)</td>
<td>15.2 (6.6)</td>
</tr>
<tr>
<td>Total parasitism&lt;sup&gt;c&lt;/sup&gt;</td>
<td><em>B. bassiana</em></td>
<td>19.0 (9.8) NS</td>
<td>34.5 (7.5) NS</td>
</tr>
<tr>
<td></td>
<td>Pyrethrin</td>
<td>20.0 (4.9)</td>
<td>35.0 (7.4)</td>
</tr>
</tbody>
</table>

Data arcsine (square root) transformed for analysis. NS = *P* > 0.05.

<sup>a</sup> Depending upon the farm and facility, pre-spray period ranged from 18 April to 18 May, 2003; spray period ranged from 07 May to 15 June, 2003; post-spray period ranged from 12 June to 28 July, 2003.

<sup>b</sup> Successful parasitism defined as the percentage of sentinel puparia from which an adult parasitoid was recovered.

<sup>c</sup> Total parasitism defined as the percentage of sentinel puparia from which an adult fly did not emerge.

Alas such as pyrethrin sprays, which is in line with integrated fly management recommendations (Kaufman et al., 2000). Geden et al. (1995) documented similar virulence for house flies and the house fly parasitoid *M. rap- tor* utilizing two house fly-collected strains of *B. bassiana*. Furthermore, they raise important concerns about the impact on beneficial arthropods of broadcast applications of *B. bassiana* conidia. We are uncertain as to the reason for the lower-than-expected house fly parasitism observed in this study; however, parasitism levels in *B. bassiana*-treated facilities were not significantly different from the pyrethrin-treated facilities (*F* = 1.51; *df* = 1, 12; *P* ≤ 0.243).

Arthropods collected from the manure core samples are presented in Table 1. Larval house fly numbers were quite high in all facilities during the pre-spray and spray period; however, larval numbers were significantly lower in the *B. bassiana*-treated facilities compared with the pyrethrin-treated facilities during the spray period. Although nearly threefold more larvae were recovered from pyrethrin-treated facilities, significant differences were not observed between facilities during the post-spray period.

*Carcinops pumilio* (Erichson) is the most important beetle predator of larval house flies in New York poultry facilities. The number of adult *C. pumilio* collected per sample during the post-spray period was 27.4 and 13.5 in *B. bassiana* and pyrethrin-treated facilities, respectively. Similarly, *C. pumilio* larvae were also found in greater abundance in the *B. bassiana*-treated facilities, 36.4 and 15.4, respectively. Although significant differences were not detected, more than twice as many *C. pumilio* adults and larvae were found in the *B. bassiana*-treated facilities as that observed in pyrethrin-treated facilities.

Darkling beetles, known omnivores, tunnel in and help to dry manure piles; however, they are also considered a pest and their presence is discouraged as their larvae destroy poultry facility support structures and building insulation. Adult and larval darkling beetles also prey on *C. pumilio* larvae (Watson et al., 2001). Darkling beetle adults and larvae were at higher numbers in *B. bassiana*-treated facilities than in the pyrethrin-treated facilities. This phenomenon was an unanticipated result and was outside the parameters of the study.

Examining house fly breeding potential provides a basis for production of additional adult house flies and an expectation as to which direction fly numbers may progress (Table 1). This method also documents the great potential of caged-layer, high-rise poultry facilities in production of house flies and the importance of an integrated approach to managing all life stages of the house fly. Initially, house fly breeding was observed in all areas of the manure pile. Subsequently, breeding retreated toward the peak area. In *B. bassiana*-treated facilities, breeding decreased from approximately 98% of the manure pile in the pre-spray period to 25% of the pile in the post-spray period. In the pyrethrin-treated facilities, breeding was also extremely high in the pre-spray period (>97%). However, unlike that observed the *B. bassiana*-treated facilities, the percentage of the manure with active breeding in pyrethrin-treated facilities was never below 54% during any study period.

House flies were abundant in the no-*Beauveria* facilities requiring many pyrethrin applications. Trends in house fly adult and larval activity are presented in Figs. 1A and B. Fly development progressed at similar rates during the pre-treatment period in all facilities validating the paired-facility requirement of this study. However, it is clear that the *B. bassiana* facilities had lower adult and larval activity after treatment initiation. Both treatment systems resulted in similar levels of fly activity at the conclusion of the study; however, it is important to understand that the critical time period for fly management is during the initial outbreak of house fly activity, typically during weeks 4–8, when an overwhelming number of flies can result in poor neighbor relations and potential lawsuits. With respect to controlling adult house flies, the *B. bassiana* + IPM treatment exceeded the control observed with the currently available best management program, pyrethrin + IPM.
3.2. Potential impacts on the system

The design of the poultry facilities and their management were similar; however, some contributing conditions should be discussed. High humidity levels uncommon in upstate New York in May and June precipitated wetter manure than is typically observed. In fact, the manure drying fans at Farm A were not effective in reducing manure moisture levels until late in the study. Additionally, producers typically remove manure in March when temperatures are much colder thus allowing the manure to accumulate, congregate, and dry prior to warm temperatures, resulting in reduced fly levels. Because of the need in this study for all facilities to begin manure accumulation at the same time, producers altered their normal operations and manure accumulation began in late April. Had the study started earlier, it is highly likely that the manure pack would have dried more quickly and fly numbers dropped more rapidly in response to treatments.

Poultry producers throughout the United States have been conditioned over the years to those fast acting pyrethrin and pyrethroid insecticides to manage house flies in their facilities. The proper use of B. bassiana will undoubtedly require an educational program to introduce producers to the concept of slow-acting fungal pathogen-based fly management. Managers at both farms were initially leery of the B. bassiana applications and were quite concerned with the off-farm house fly movement prior to the suppression effect. In the case of Farm B, the manager was so concerned that he considered removing the farm from the study. However, when it was pointed out that the pyrethrin-treated house had equal to or greater fly populations, he agreed to hold off treatment until the following week, by which time fly suppression had begun. This incident underscores a learning point as a product such as this moves through the research/development stage and into marketing. If producers wait for a fly outbreak before beginning B. bassiana applications, they must be fully aware of the comparatively slow-acting nature of this material. It should prove more useful for a product such as this to be used in a proactive method, rather than waiting until a fly problem develops as we specifically did in order to document the effectiveness of the product. Proactive research studies are most certainly needed.

Pesticide resistance development by the house fly has been considerable (Kaufman et al., 2001b; Meyer et al., 1987; Scott et al., 2000). Essentially, pyrethrin space sprays and baits are now the only effective materials that can be utilized during a caged-layer flock cycle, which is the only time flies are a problem in facilities (Scott et al., 2000). Additionally, the poultry industry has already lost and will continue to lose many of the currently registered pesticides for fly management due to the implementation of the Food Quality Protection Act. These two driving issues demand that new cost-effective pest management tools be developed and introduced to the poultry industry.

Geden et al. (1995) reported that adult house flies infected by B. bassiana died within 5 days of exposure. However, in the very extreme microbial environment that occurs in poultry facilities, few flies actually exhibit signs of infection such as post-emergence of the fungus followed by conidiation on the surface of the cadavers, even if dead adults are subsequently placed in chambers with adequate humidity. Various bacteria and other fungi such as Aspergillus spp. usually colonize the cadaver too quickly to allow for development of Beauveria post-mortem. Thus, it has not been possible to initiate epizootics after treatments in poultry facilities.

Typically, this strain of B. bassiana requires 2–5 days to kill flies, therefore, the adult numbers do not drop from a visible perspective as quickly as that observed with a pyrethrin treatment (J.J.A. pers. comm.). However, when examined as an overall pest management program, the achievement of actual fly control is enhanced with B. bassiana as a tool because the producer actually reaches a point where they can stop treatments. This is not the case when a traditional adulticide program is followed and producers are often faced with repeated applications to suppress house flies. This is pos-

![Image](image-url)
sible due to two phenomena: (1) fungal treatments are applied in the manure pits where the flies are breeding and emerging, allowing control prior to mating and first egg lay and (2) the use of the product does not impede the development of coleopteran beneficial insect programs and may, in fact, create an environment for preda
cicious natural enemies of flies to thrive. An appreciable increase of this beneficial impact on fly control may be accomplished by allowing naturally occurring prey (fly eggs and prey mites) during early manure accumulation thus allowing for rapid increases in fly natural enemy populations. However, by slowly reducing food availability as both the manure dries and fly oviposition decreases, the overall impact that natural enemies have on immature fly survival increases, resulting in enhanced fly control, as observed in this study. In a traditional pesticide-based fly management program, a proportion of the beneficial population is always impacted at each pesticide application. Additionally, house fly larval activity may in fact increase suitable larval habitat for the next generation by churning up the manure and by drawing moisture from within the manure pile, thus keeping the surface moist. Therefore, it is also likely that the decrease in house fly oviposition, achieved by killing young adult female flies with \textit{B. bassiana} applications, and subsequent lower larval numbers in manure will enhance manure drying and result in lower fly numbers.

Producers currently have the ability to purchase and release house fly predators and parasitoids, nurture their own fly natural enemies, and utilize cultural and physical control strategies (Axtell, 1999; Kaufman et al., 2000). However, until the development of balance by Jabb of the Carolinas, successful fungal pathogen utilization has not been possible on a scale suitable to commercial egg production. The development of \textit{B. bassiana} as an effective tool against the house fly is a critical component to a significant challenge that has confounded poultry producers: how to kill large numbers of adult house flies without using residual premise pesticides. Now an integrated fly management program can include the full complement of tools targeting all life stages of the house fly while preserving and protecting fly natural enemies resulting in maintenance of fly populations below maximally accepted levels in poultry facilities.

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