# Horizontal transmission of *Metarhizium anisopliae* (Hypocreales: Clavicipitacea) and the effects of infection on oviposition rate in laboratory populations of *Musca domestica* (Diptera: Muscidae).

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## ABSTRACT

**BACKGROUND**

Effective control of house fly, *Musca domestica* (L.), populations currently relies on the use of chemical insecticides in most situations. Entomopathogenic fungi such as *Metarhizium anisopliae* (Metschn.) Sorokin may provide an alternative to chemicals and their efficacy may be enhanced by autodissemination amongst flies. This study assessed the capacity of *M. anisopliae* for transmission between adult *M. domestica* and the effects of infection on the fecundity of females.

**RESULTS**

*Metarhizium anisopliae* was transmitted between adult *M. domestica* flies with 91.67% - 100% mortality resulting across the three ratios of infected: non-infected flies tested (1:2, 1:5, 1:10). The mean lethal time ($LT_{50}$) for female recipients mixed with infected male donor flies at the three ratios were 3.95, 4.79 and 5.65 days, respectively, whereas for male recipients mixed with infected female donors at the same ratios the $LT_{50}$ were 4.98, 5.98 and 7.44 days, respectively. Infection with *M. anisopliae* significantly reduced the reproductive capacity of female flies during the first four days of infection with 25% less eggs oviposited by infected flies than those that were uninfected.

**CONCLUSION**

Autodissemination among house flies and reduction in oviposition in the early stages of infection could contribute significantly to the effectiveness of *M. anisopliae* used in biocontrol programs.

**KEYWORDS**   house fly, biological control, mycoinsecticide, entomopathogenic fungi

## 1. INTRODUCTION

House flies, *Musca domestica* (L.), are ubiquitous cosmopolitan pests of serious agricultural and public health importance. The nature of their breeding sites (primarily animal faeces and other decaying organic material), attraction to food and animal feed and their mode of feeding make house flies important mechanical vectors for more than one hundred pathogens including the enterohaemmorhagic *Escherichia coli* strain O157:H7[1, 2]. Currently, the control of house fly populations is heavily reliant on the use of chemical insecticides in both spray and bait formulations[3]. Excessive use of chemical insecticides has resulted in populations of *M. domestica* becoming resistant to almost all classes of insecticides used against them, including; organophosphates, carbamates, pyrethroids, insect growth regulators[4] as well as relatively new insecticides including spinosad, imidacloprid and nithiazine[5, 6]. In addition, extended occupational exposure to some classes of chemical insecticides may have adverse health effects[7, 8]. These concerns have led to intensive research towards safer alternatives for house fly management.

Mycoinsecticide formulations based on entomopathogenic fungi provide a more user and environmentally friendly alternative to conventional chemical insecticides. The predominant fungi used in this manner are *Metarhizium anisopliae* (Metschn.) Sorokin and *Beauveria bassiana* (Bals. – Criv.) Vuill., but formulations of other fungi such as *Isaria fumosorosea* (Wize) and *Beauveria brongniartii* (Sacc.) Petch have also shown promise[9]. The susceptibility of *M. domestica* to several species of entomopathogenic fungi is well documented[10-12] and multiple studies have indicated that isolates of *M. anisopliae* can be particularly virulent[13, 14], but a mycoinsecticide product for the control of house flies based on *M. anisopliae* is yet to be commercially developed. *Metarhizium anisopliae* (BRIP #42412) has shown significant potential for house fly mycoinsecticide development as it has shown high virulence to *M. domestica* and exhibited high yield and germination rate through multiple production runs (Leemon DM, unpublished). These qualities indicate the potential for development of this strain in a mycoinsecticide formulation for direct application.

Previous research has suggested that it may be possible to develop mycoinsecticides and host-release strategies that utilize host behaviour to assist autodissemination of the fungal pathogen through the population. Strategies such as this would minimise non-target mortality resulting from the non-specific nature of entomopathogenic fungi[15] and may increase the efficacy of the mycoinsecticide by promoting persistence of the fungus in the population post-treatment. Previous studies have demonstrated that entomopathogenic fungi can be used concurrently with parasitoid wasps, but strain differences can result in high levels of wasp mortality[16, 17]. Reducing the non-target mortality would also facilitate the use of the fungus with other biocontrol agents and natural enemies. Transmission of *M. anisopliae* among host insects[18-23], including *M. domestica*[24, 25], has previously been demonstrated but rates of transmission and the effects of infection can vary between strains[10, 14, 26].

Reductions in the reproductive capacity of insects infected with *Metarhizium* isolates have been demonstrated in multiple insect species[27-30]. However, there is limited research on the reproductive effects of *M. anisopliae* infection in *M. domestica*[31, 32]. Unlike chemical insecticides, entomopathogenic fungi are relatively slow-killing, which could potentially allow for insect reproduction to occur prior to death. A reduction in the reproductive potential of infected female *M. domestica* may add to the effect of mycoinsecticide formulations applied both directly and through host release strategies. This study assessed the ability of *M. anisopliae* to autodisseminate within laboratory populations of *M. domestica* and determined the effects of infection on the fecundity of females prior to death.

## 2. EXPERIMENTAL METHODS

### 2.1 Insect cultures

*Musca domestica* used in this study were reared in a controlled environment room at constant conditions of 27±2°C, 67±5% relative humidity (RH) and a photoperiod of 12:12 (light:dark) hours. Larvae were reared on a wheat bran, alfalfa meal (milled lucerne) and cornmeal medium (2:1:0.5 by

weight, water to moisten). Adult flies were given a protein feed consisting of skimmed milk powder, wheat bran and alfalfa meal (2:1:0.5 by weight, water to moisten) for egg development. Gravid flies were provided with a small dark vial containing approximately 30g of egg laying medium (1:1 mix of old and new larval media) for a period of 6 hours for oviposition. Approximately 0.7g of eggs were transferred to a 1L plastic container containing larval medium to initiate the next generation.

## 2.2 Fungal isolate and spore production

The *Metarhizium anisopliae* M16 isolate (BRIP #42412) used in this study was taken from the Queensland Department of Agriculture and Fisheries (DAF) entomopathogenic fungal culture collection housed at the Ecosciences Precinct (ESP) Dutton Park. This isolate was originally obtained from soil samples collected in Queensland, maintained on potato dextrose agar (PDA) (BD Difco™, Franklin Lakes, NJ, USA) slopes held at 4°C and -20°C. Production of M16 conidia was completed via a biphasic process with a liquid culture grown to inoculate solid media. The liquid culture consisted of sterile yeast peptone broth inoculated with conidia scraped from 14 day cultures on oatmeal agar (BD Difco™). Liquid cultures were grown for 5 days at 28°C in an orbital shaker at 120rpm. Mushroom spawn culture bags containing 1.5kg sterilised rice were each inoculated with 150ml of the liquid culture and incubated for seven days at 28°C on wire racks after which the solid cultures were broken up and incubated for a further 14 days. Bags were then opened and left to air dry for 4-5 days at 19°C in a de-humidified room.

## 2.3 Horizontal transmission of *Metarhizium anisopliae* in adult *Musca domestica*

Newly emerged flies (1-2 days post-eclosion, not protein fed) were randomly selected from the laboratory colony and anaesthetised with $CO_2$, then sexed by external morphology and transferred to holding containers. A subsample of flies for infection (donor flies) was then randomly selected from each of the holding containers and marked with nail polish for identification purposes. The donor flies were transferred to a ventilated assay container (70mm h × 80mm dia.) holding 10g of

well sporulated *M. anisopliae* rice substrate (approximately $1.2 \times 10^{10}$ conidia/g), in which they were allowed to walk for 5min. Three replicates each of 1, 2 and 5 infected donor flies of each sex were then transferred to clean, assay containers for one hour. Ten uninfected flies of the opposite sex were then introduced to each container. Control donor flies were similarly marked with nail polish and added to ventilated assay containers at the same ratios to recipient flies. Approximately 2g of sugar and *ad libitum* water were provided for each container. Assay containers were held in an incubator at 27±2°C, 67±5% relative humidity (RH) and a photoperiod of 12:12 light:dark (L:D) hours for 14 days and mortality was recorded every 24 hours. Dead flies were removed from the assay containers then surface sterilised in 70% ethanol and plated on 1.5% water agar. Plated flies were incubated at 27±2°C for 7 days then checked for mycosis. Two replicates of this assay were conducted.

**2.4 Effects of *Metarhizium anisopliae* infection on the rate of oviposition in *Musca domestica* females**

Bioassays were conducted in 6 mesh cages (600 x 600 x 600mm) inside a controlled environment room (27°C, 65% RH and 12:12 L:D). One hundred, unsexed, *M. domestica* adults (3-4 days post-eclosion, protein fed) were placed within each cage. After one hour, 10g of *M. anisopliae* spores and rice substrate (approximately $1.2 \times 10^{10}$ conidia/g), held within the base of a 9mm Petri dish, were placed in three of the cages for 24 hours. To collect eggs, all cages were supplied with egg laying medium (described in section 2.1) inside a mesh bag that was placed within a small vial for 6 hours each day, over a 7 day period. The eggs were rinsed into Falcon tubes with deionised water and shaken gently to break up egg clumps. They were then transferred to black filter paper, allowed to dry for 30mins and weighed. To calibrate the egg number-to-mass relationship a modified version of the method described by Acharya *et al.*[32] was used. Fifteen subsamples of eggs from the laboratory culture, within the 100-1000 egg range, were individually counted and weighed. The egg number to mass relationship was described by the following regression equation: Total eggs = (17.955 × egg

mass (mg)) – 28.585; $R^2$ = 0.956. Fly mortality was recorded daily before the oviposition medium was supplied and the dead flies were sexed so that the daily egg production per female could be calculated. A fresh protein feed, as described in section 2.1, was provided nightly to promote maximum egg production. This assay consisted of two replicates.

**2.5 Statistical analysis**

Survival data for the autodissemination experiment were analysed using probit analysis. Abbott's formula[33] was used to adjust for the control mortality. Total mortality and infection rates were analysed using a generalised linear model (GLM) for a binomial distribution with the logit-link function. Probabilities from the GLM were estimated using deviance ratios. Means were estimated via back-transformation of the link function and the differences between means were compared using Fisher's LSD test. Mean oviposition rate per female was analysed using a repeated measures analysis of variance (ANOVA). This forms an approximate split-plot analysis of variance (split for time). The Greenhouse-Geisser epsilon correction factor was used to adjust for temporal autocorrelation before the probabilities were calculated. All data analyses were performed using Genstat[34].

3. **RESULTS**

**3.1 Horizontal transmission of *Metarhizium anisopliae* in adult *Musca domestica***

The total mortality and infection rate of *M. anisopliae* in recipient flies was significantly greater in flies exposed to infected donors than in the control groups across all sex ratios ($P < 0.001$) (Table 1). There were no differences in the total mortality of the control groups except the total mortality of recipient males was significantly higher than that of the recipient females for the 1:2 proportion, 16.67% and 5.00%, respectively. No *M. anisopliae* infection was detected within the control groups, however, significant infection was detected in the recipient flies of all treatment groups ($P < 0.001$). In the treatment groups, the total mortality of recipient males in the 1:10 proportion was

significantly lower than all other ratios ($P < 0.001$). The infection rate of dead recipient flies within

the treatment groups ranged from 66.10-83.33% but there was no significant difference in infection

rate between the groups (Table 1).

The mean lethal time ($LT_{50}$) of recipient *M. domestica* exposed to infected donors was significantly

different between treatment groups ($P < 0.001$). Both sex ($P < 0.001$) and donor proportion ($P <

0.001$) had a significant effect on the adjusted mortality of the recipient flies. The $LT_{50}$ estimates of

female recipients at 1:2, 1:5 and 1:10 proportions were 3.95, 4.79 and 5.65, days respectively, while

the $LT_{50}$ estimates of male recipients at the same donor proportions were 4.98, 5.98 and 7.44 days,

respectively (Table 2). The effect of sex on the mortality of the recipient flies did not differ over time

($P = 0.076$) but the effect of donor proportion was significantly different over time ($P < 0.001$).

**3.2 Effects of *Metarhizium anisopliae* infection on the rate of oviposition in *Musca domestica***

**females**

The exposure of gravid *M. domestica* females to *M. anisopliae* significantly reduced the overall rate

of oviposition of these females during the first four days post-exposure ($P = 0.033$) (Fig. 1). There

were no significant differences in oviposition rate between the control and treatment females during

the first two days post-exposure, but oviposition by exposed females was significantly lower than

the control females on days three and four ($P < 0.05$). As expected, the mortality rate of exposed

females was significantly higher than that of the controls ($P < 0.001$). The mean cumulative mortality

of exposed females was 64.58% and 96.53% at four and five days, respectively (Fig. 1). Thus, only

data from 1-4 days post-exposure was used for comparative analysis of oviposition rates. The control

mortality reached a cumulative average of 9.92% by day seven.

## 4.  DISCUSSION

The *Metarhizium anisopliae* isolate assessed in this study was determined to be readily transmissible

between adult *Musca domestica*. The present study demonstrated 91.67-100.00% mortality via

transmission of infection across all treatment groups with only the total mortality of recipient males at the lowest donor proportion (1:10) being significantly different from the other treatment groups. These results are similar to Cárcamo et al.[24] with M. domestica and Toledo et al.[35] and Quesada-Moraga et al.[23] with Tephritidae (Diptera) as hosts. However, Cárcamo et al.[24] observed a significant effect of sex on the total mortality caused by the horizontal transmission of their M. anisopliae isolate. The study showed transmission of the fungus from female donors to male recipients resulted in a mean mortality of 55% as opposed to the 100% mortality observed in female recipients. Comparisons between the present study and Cárcamo et al.[24] are however difficult to make due to the difference in the inoculation method. Dry formulations of conidia are generally better adapted for use in autodissemination strategies than liquid ones[36] and, therefore, the differences in the total mortality of the male recipient flies observed between the two studies could potentially be attributed to the present study using a dry conidia substrate to inoculate flies.

The lower survival time of female than male M. domestica infected with M. anisopliae through horizontal transmission is in accordance with the results of Quesada-Moraga et al.[23] and Cárcamo et al.[24]. Quesada-Moraga et al.[23] observed that the aedeagus of Ceratitis capitata (Wiedemann) (Diptera: Tephritidae) had become infected with conidia after the flies were inoculated with the fungus. Cárcamo et al.[24] suggested that this is also likely to occur in M. domestica and that the lower survival time in females infected via transmission from males is due to the increased pathogenic load transmitted to the females during copulation. Leemon and Jonsson[37] observed no evidence of M. anisopliae infection through the external cuticle of Lucilia cuprina (Wiedemann) (Diptera: Calliphoridae) when assessing exposed individuals via scanning electron microscopy (SEM), however infection was observed in the buccal cavity of the fly. This suggests that M. anisopliae infection of some dipteran species may occur more readily if conidia can germinate internally. The lower survival time in female M. domestica infected with M. anisopliae may also be due to a post-mating reduction in immune defence[38-40]. Only newly emerged (1-2 day old) flies were used in the transmission assay, however, sufficient mating may have occurred during this time and caused the difference in survival

time. The higher efficacy of male to female transmission may lend itself to strategies regarding the release of infected individuals as male *M. domestica* mate more than females[41]. These traits may be able to be exploited for autodissemination strategies without the release of females that can actively contribute to the growth of the population.

Survival time of the recipient flies in the horizontal transmission assay was determined to be dependent on the proportions of infected to uninfected flies, however, total mortality was not. The $LT_{50}$ of recipient flies was significantly different between the proportions such that a greater number of infected donor flies resulted in faster transmission and subsequent mortality of the recipient flies. This suggests that at lower donor proportions than those tested here; the total mortality of recipient flies is likely to decrease. Quesada-Moraga *et al.*[23], for example, did observe a lower total mortality in recipient *C. capitata* females, with 62.5% and 42.5% mortality at 1:10 and 1:20 proportions, respectively. In order to be successful, strategies using autodissemination of *M. anisopliae* to control *M. domestica* populations would require an initial release of a relatively large number of infected flies.

Exposure to *M. anisopliae* significantly reduced the oviposition rate of female *M. domestica* with a 53-98% reduction in oviposition from 3-4 days after treatment. Similar observations have been made in previous studies using different inoculation methods[25, 32]. *Metarhizium anisopliae* causes muscle paralysis (tetany) followed by muscle weakness in infected insects prior to death[42], which may explain the effect of mycotic infection on oviposition rate of the females. This pre-lethal effect further supports the use of myco-insecticide formulations for both direct application and autodissemination strategies as it demonstrates additional, population reducing effects aside from direct mortality.

## 5. ACKNOWLEDGEMENTS

## 6. REFERENCES

1.  Ahmad A, Nagaraja TG and Zurek L, Transmission of *Escherichia coli* O157: H7 to cattle by house flies. *Preventive Veterinary Medicine*; **80**(1): 74-81 (2007).

2.  Ghosh A and Zurek L, Fresh steam-flaked corn in cattle feedlots is an important site for fecal coliform contamination by house flies. *Journal of Food Protection*; **78**(3): 567-572 (2015).

3.  Axtell RC and Arends JJ, Ecology and management of arthropod pests of poultry. *Annual Review of Entomology*; **35**: 101-126 (1990).

4.  Keiding J, Review of the global status and recent development of insecticide resistance in field populations of the housefly, *Musca domestica* (Diptera: Muscidae). *Bulletin of Entomological Research*; **89**: S9-S67 (1999).

5.  Wang Q, Li M, Pan J, Di M, Liu Q, Meng F, Scott JG and Qiu X, Diversity and frequencies of genetic mutations involved in insecticide resistance in field populations of the house fly (*Musca domestica* L.) from China. *Pesticide Biochemistry and Physiology*; **102**(2): 153-159 (2012).

6.  Scott JG, Leichter CA, Rinkevihc FD, Harris SA, Su C, Aberegg LC, Moon R, Geden CJ, Gerry AC, Taylor DB, Byford RL, Watson W, Johnson G, Boxler D and Zurek L, Insecticide resistance in house flies from the United States: resistance levels and frequency of pyrethroid resistance alleles. *Pesticide Biochemistry and Physiology*; **107**(3): 377-384 (2013).

7.  Rizzati V, Briand O, Guillou H and Gamet-Payrastre L, Effects of pesticide mixtures in human and animal models: an update of the recent literature. *Chemico-Biological Interactions*; **254**: 231-246 (2016).

8.  Campos E and Freire C, Exposure to non-persistent pesticides and thyroid function: a systematic review of epidemiological evidence. *Int J Hyg Environ Health*; **219**(6): 481-497 (2016).

9.  Faria MR and Wraight SP, Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. *Biological Control*; **43**: 237-256 (2007).

10. Anderson RD, Bell AS, Blanford S, Paaijmans KP and Thomas MB, Comparative growth kinetics and virulence of four different isolates of entomopathogenic fungi in the house fly (*Musca domestica* L.). *Journal of Invertebrate Pathology*; **107**(3): 179-184 (2011).

11. Lopez-Sanchez J, Cruz-Vazquez C, Lezama-Gutierrez R and Ramos-Parra M, Effect of entomopathogenic fungi upon adults of *Stomoxys calcitrans* and *Musca domestica* (Diptera: Muscidae). *Biocontrol Science and Technology*; **22**(8): 969-973 (2012).

12. Farooq M and Freed S, Infectivity of housefly, *Musca domestica* (Diptera: Muscidae) to different entomopathogenic fungi. *Brazilian Journal of Microbiology*; **47**(4): 807-816 (2016).

13. Barson G, Renn N and Bywater AF, Laboratory evaluation of 6 species of entomopathogenic fungi for the control of the house fly (*Musca domestica* L.), a pest of intensive animal units. *Journal of Invertebrate Pathology*; **64**(2): 107-113 (1994).

14. Mishra S, Kumar P, Malik A and Satya S, Adulticidal and larvicidal activity of *Beauveria bassiana* and *Metarhizium anisopliae* against housefly, *Musca domestica* (Diptera: Muscidae), in laboratory and simulated field bioassays. *Parasitology Research*; **108**(6): 1483-1492 (2011).

15. Goettel MS, Poprawski JD, Vandenberg JD, Li Z and Roberts DW. Safety to nontarget invertebrates of fungal biocontrol agents. In *Safety of microbial insecticides*, ed. by Laird M, Lacey LA and Davidson EW. CRC: Boca Raton, FL, pp. 209-231 (1990).

16. Geden CJ, Rutz DA and Steinkraus DC, Virulence of different isolates and formulations of *Beauveria bassiana* for house flies and the parasitoid *Muscidifurax raptor*. *Biological Control*; **5**(4): 615-621 (1995).

17. Nielsen C, Skovgard H and Steenberg T, Effect of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) on survival and reproduction of the filth fly parasitoid, *Spalangia cameroni* (Hymenoptera: Pteromalidae). *Environmental Entomology*; **34**(1): 133-139 (2005).

18. Kaaya GP and Okech MA, Horizontal transmission of mycotic infection in adult tsetse, *Glossina morsitans morsitans*. *Entomophaga*; **35**(4): 589-600 (1990).

19. Kaakeh W, Reid BL and Bennett GW, Horizontal transmission of the entomopathogenic fungus *Metarhizium anisopliae* (Imperfect fungi: Hyphomycetes) and hydramethylnon among German cockroaches (Dictyoptera: Blattellidae). *Journal of Entomological Science*; **31**(4): 378-390 (1996).

20. Furlong MJ and Pell JK, Horizontal transmission of entomopathogenic fungi by the diamondback moth. *Biological Control*; **22**(3): 288-299 (2001).

21. Quesada-Moraga E, Santos-Quiros R, Valverde-Garcia P and Santiago-Alvarez C, Virulence, horizontal transmission, and sublethal reproductive effects of *Metarhizium anisopliae* (Anamorphic fungi) on the German cockroach (Blattodea: Blattellidae). *Journal of Invertebrate Pathology*; **87**(1): 51-58 (2004).

22. Scholte EJ, Knols BGJ and Takken W, Autodissemination of the entomopathogenic fungus *Metarhizium anisopliae* amongst adults of the malaria vector *Anopheles gambiae* ss. *Malaria Journal*; **3:** (2004).

23. Quesada-Moraga E, Martin-Carballo I, Garrido-Jurado I and Santiago-Alvarez C, Horizontal transmission of *Metarhizium anisopliae* among laboratory populations of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). *Biological Control*; **47**(1): 115-124 (2008).

24. Carcamo MC, Felchicher F, Duarte JP, Bernardi E and Ribeiro PB, Horizontal Transmission of *Beauveria bassiana* (Hypocreales: Cordycipitaceae) and *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) in *Musca domestica* (Diptera: Muscidae). *Journal of Economic Entomology*; **108**(4): 1579-1586 (2015).

25. Garcia-Munguía CA, Reyes-Villanueva F, Rodriguez-Perez MA, Cortez-Madrigal H, Acosta-Ramos M, Ibarra-Juárez LA, Velázquez-Machuca MA, Silva-García JT, Rebollar-Plata M and Garcia-Munguía AM, Autodissemination of *Metarhizium anisopliae* and *Beauvaria bassiana* in *Musca domestica* L. results in less oviposition and short gonotrophic cycle. *Southwestern Entomologist*; **40**(3): 519-529 (2015).

26. Lecuona RE, Turica M, Tarocco F and Crespo DC, Microbial control of *Musca domestica* (Diptera: Muscidae) with selected strains of *Beauveria bassiana*. *Journal of Medical Entomology*; **42**(3): 332-336 (2005).

27. Migiro LN, Maniania NK, Chabi-Olaye A, Wanjoya A and Vandenberg J, Effect of infection by *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) on the feeding and oviposition of the pea leafminer *Liriomyza huidobrensis* (Diptera: Agromyzidae) on different host plants. *Biological Control*; **56**(2): 179-183 (2011).

28. Mouatcho JC, Koekemoer LL, Coetzee M and Brooke BD, The effect of entomopathogenic fungus infection on female fecundity of the major malaria vector, *Anopheles funestus*. *African Entomology*; **19**(3): 725-729 (2011).

29. Sookar P, Bhagwant S and Allymamod MN, Effect of *Metarhizium anisopliae* on the fertility and fecundity of two species of fruit flies and horizontal transmission of mycotic infection. *Journal of Insect Science*; **14:** (2014).

30. Cossentine J, Robertson M and Buitenhuis R, Impact of acquired entomopathogenic fungi on adult *Drosophila suzukii* survival and fecundity. *Biological Control*; **103:** 129-137 (2016).

31. Bywater AF, Barson G, Renn N and Bcpc. The potential of oil-based suspensions of *Metarhizium anisopliae* conidia for the control of the housefly (*Musca domestica*), a pest of intensive animal units. In *Brighton Crop Protection Conference - Pests and Diseases - 1994, Vols 1-3*, pp. 1097-1102 (1994).

32. Acharya N, Rajotte EG, Jenkins NE and Thomas MB, Potential for biocontrol of house flies, *Musca domestica*, using fungal biopesticides. *Biocontrol Science and Technology*; **25**(5): 513-524 (2015).

33. Abbott WS, A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*; **18:** 265-267 (1925).

34. Payne R, Murray D and Harding S. An Introduction to the Genstat Command Language (18th Edition). VSN International: Hemel Hempstead, Hertfordshire, UK (2015).

35. Toledo J, Campos SE, Flores S, Liedo P, Barrera JF, Villasenor A and Montoya P, Horizontal transmission of *Beauveria bassiana* in *Anastrepha ludens* (Diptera: Tephritidae) under laboratory and field cage conditions. *Journal of Economic Entomology*; **100**(2): 291-297 (2007).

36. Meadow R, Vandenberg JD and Shelton AM, Exchange of inoculum of *Beauveria bassiana* (Bals.) Vuill. (Hyphomycetes) between adult flies of the cabbage maggot *Delia radicum* L. (Diptera : Anthomyiidae). *Biocontrol Science and Technology*; **10**(4): 479-485 (2000).

37. Leemon DM and Jonsson NN, Comparative studies on the invasion of cattle ticks (*Rhipicephalus* (*Boophilus*) *microplus*) and sheep blowflies (*Lucilia cuprina*) by *Metarhizium anisopliae* (Sorokin). *Journal of Invertebrate Pathology*; **109**(2): 248-259 (2012).

38. Siva-Jothy MT, Tsubaki Y and Hooper RE, Decreased immune response as a proximate cost of copulation and oviposition in a damselfly. *Physiological Entomology*; **23**(3): 274-277 (1998).

39. Rolff J and Siva-Jothy MT, Copulation corrupts immunity: A mechanism for a cost of mating in insects. *Proceedings of the National Academy of Sciences of the United States of America*; **99**(15): 9916-9918 (2002).

40. Short SM and Lazzaro BP, Female and male genetic contributions to post-mating immune defence in female *Drosophila melanogaster*. *Proceedings of the Royal Society B-Biological Sciences*; **277**(1700): 3649-3657 (2010).

41. Murvosh CM, Smith CN and Labrecque GC, Effect of 3 chemosterilants on house fly longevity + sterility. *Journal of Economic Entomology*; **57**(1): 89-93 (1964).

42. Samuels RI, Reynolds SE and Charnley AK, Calcium channel activation of insect muscle by destruxins, insecticidal compounds produced by entomopathogenic fungus *Metarhizium anisopliae*. *Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology*; **90**: 403-412 (1998).

## 7. TABLES

**Table 1:** Mean total mortality and observed mycosis in dead recipient house flies, *Musca domestica*, exposed to different proportions of donor flies infected with *Metarhizium anisopliae*. Means within the same column followed by the same letter are not significantly different. Mortality data was recorded to 14 days post treatment.

| Proportion | Group | Donor Sex | Mortality (mean ± SE) | |
|---|---|---|---|---|
| | | | Total (%) | Mycosis (%) |
| 1:2 | Control | ♂ | $5.00 \pm 2.81^a$ | $0.00 \pm 0.18^a$ |
| | | ♀ | $16.67 \pm 4.81^b$ | $0.00 \pm 0.08^a$ |
| | Treatment | ♂ | $100.00 \pm 0.02^d$ | $83.33 \pm 4.81^b$ |
| | | ♀ | $100.00 \pm 0.02^d$ | $76.67 \pm 5.46^b$ |
| 1:5 | Control | ♂ | $6.67 \pm 3.22^{ab}$ | $0.00 \pm 0.15^a$ |
| | | ♀ | $10.00 \pm 3.87^{ab}$ | $0.00 \pm 0.12^a$ |
| | Treatment | ♂ | $98.33 \pm 1.65^{cd}$ | $66.10 \pm 6.16^b$ |
| | | ♀ | $100.00 \pm 0.02^d$ | $66.67 \pm 6.09^b$ |
| 1:10 | Control | ♂ | $8.00 \pm 3.84^{ab}$ | $0.00 \pm 0.09^a$ |
| | | ♀ | $10.00 \pm 3.87^{ab}$ | $0.00 \pm 0.12^a$ |
| | Treatment | ♂ | $100.00 \pm 0.02^d$ | $66.67 \pm 6.09^b$ |
| | | ♀ | $91.67 \pm 3.57^c$ | $76.36 \pm 5.73^b$ |

**Table 2:** Mean lethal time in days to 50% mortality ($LT_{50}$) and fiducial limits for recipient *Musca domestica* infected via horizontal transmission of *Metarhizium anisopliae* from infected donor flies of the opposite sex at different donor proportions ($n$ = 60). $LT_{50}$ values with the same letter have overlapping fiducial limits and are not considered to be significantly different.

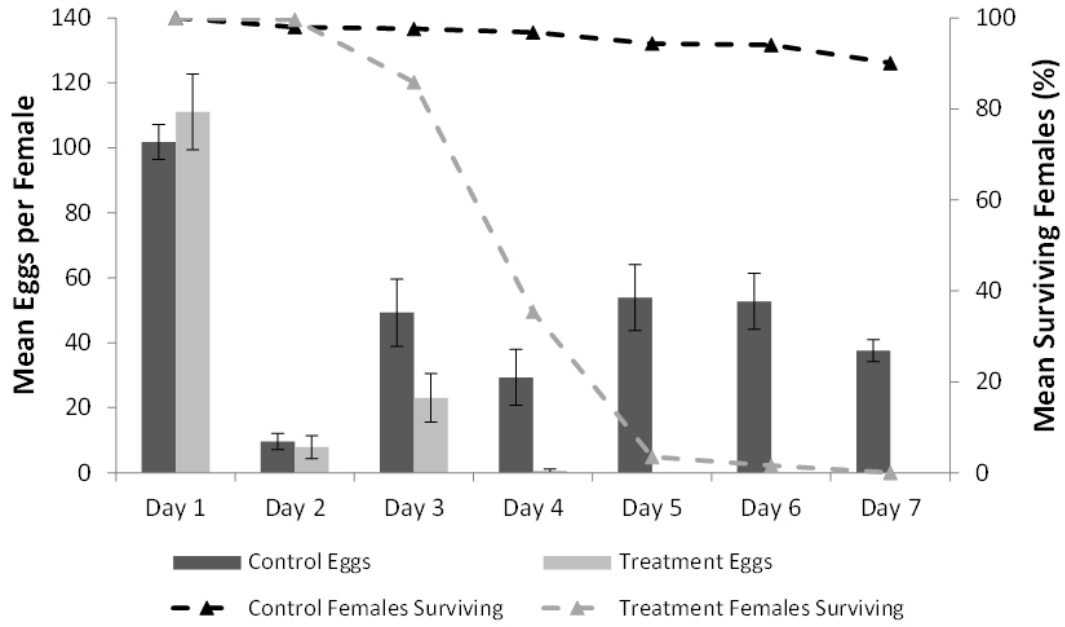| Proportion | Donor Sex | $LT_{50}$ | *t*-value | Slope ± SE | Fiducial Limits | |
|---|---|---|---|---|---|---|
| | | | | | Lower 95% | Upper 95% |
| **1:2** | ♂ | $3.95^d$ | -9.61 | $-6.61 \pm 0.69$ | 3.77 | 4.12 |
| | ♀ | $4.98^c$ | -10.67 | $-6.63 \pm 0.62$ | 4.76 | 5.19 |
| **1:5** | ♂ | $4.79^c$ | -11.74 | $-4.28 \pm 0.37$ | 4.51 | 5.07 |
| | ♀ | $5.99^b$ | -12.86 | $-6.40 \pm 0.50$ | 5.71 | 6.26 |
| **1:10** | ♂ | $5.65^b$ | -12.86 | $-5.68 \pm 0.44$ | 5.38 | 5.93 |
| | ♀ | $7.44^a$ | -14.19 | $-4.68 \pm 0.33$ | 7.03 | 7.85 |

**8. FIGURES**



Figure 1

## 9. FIGURE LEGENDS

**Figure 1:** Eggs oviposited per live *Musca domestica* female (mean ± SE) and mean percentage surviving females after

exposure to *Metarhizium anisopliae*.

## 10. GRAPHICAL ABSTRACT
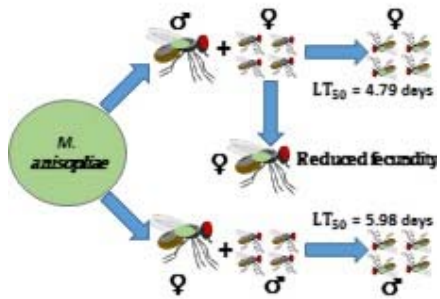


**Horizontal transmission of *Metarhizium anisopliae* (Hypocreales: Clavicipitacea) and the effects of infection on oviposition rate in laboratory populations of *Musca domestica* (Diptera: Muscidae).**

Dalton K. Baker, Steven J. Rice, Diana M. Leemon and Peter J. James*

*Metarhizium anisopliae* was readily transmitted between adult *Musca domestica* with mortality rates dependent on both sex and the ratio of donor to recipient flies. Infection also reduced female fly fecundity.