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A generalized Poisson model to predict host-seeking female *Aedes aegypti* marked by dusted *Metarhizium anisopliae*-exposed males

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ABSTRACT: We developed a biological control method directed toward *Aedes aegypti* using the release of *Metarhizium anisopliae*-contaminated males to spread the fungus to wild females. A generalized Poisson model was used to relate *Ae. aegypti* marked females (MKF) to *M. anisopliae*-exposed males (FEM). In a mark-recapture parallel arm trial, FEM release was a better predictor than unexposed male (UM) releases to forecast MKF by FEM. Total females (TF), marked males (MKM), and wild males (WM) as predictors were counted in human-landings in 15 households treated with 40 FEM each, vs 40 UM released/household/week in 15 households for eight weeks. Fit of MKF to standard, generalized Poisson (GP), and negative binomial models/arm built by TF, MKM, WM, and interactions as predictors were computed. In both arms, MKF was better modeled by GP, which in treated, all but one of the eight observed data fell within the confidence intervals predicted by the model. However, the control GP had two outliers and MKM as a single predictor. Likewise, the pseudo-R^2 measures of 95% and 46% for treated and control groups also showed that the GP with FEM was more suitable to predict MKF. It should thus be possible to use the GP model to indirectly estimate that an increase of one TF or one fungus-exposed male would increase the number of marked-females by 8% or 9%, respectively, while wild males were an irrelevant predictor to the model. ***Journal of Vector Ecology* 48 (1): 52-58. 2023.**

Keyword Index: Autodissemination, fungus-exposed males, Poisson model, *Aedes aegypti, Metarhizium anisopliae.*

INTRODUCTION

Aedes aegypti mosquitoes live in urban habitats and can potentially transmit over 22 arboviruses that annually infect up to 400 million people worldwide (Brady and Hay 2020). Its high vector competence is why it is the principal vector of the four serotypes of the dengue virus (DENV) (Liu-Helmersson et al. 2014). The ever-increasing resistance to chemicals and the resistance from local communities to the removal of *Ae. aegypti* larval containers (Campos et al. 2020) has encouraged the quest for alternatives such as *Metarhizium anisopliae*, a promising agent killing 50% of mosquitoes in four to seven days by indirect exposure (Scholte et al. 2007, Paula et al. 2008, 2011) and in seven to eight days when it is spread from males to females (Reyes-Villanueva et al. 2011). *Aedes aegypti* females exposed to *M. anisopliae* (5.96×10^7 dry conidia/cm^2) and infected with DENV-2 (fed on human blood infected with 1×10^7 plaque-forming units/ml) showed a 78% mortality, and 12% of survivors showed DENV-2 at day seven post-infection, while in single-virus infected females, the mortality dropped to 6% and in the head-viremic survivors increased up to 64%. Further, the median lethal time in single-fungal infections was 70% shorter than that of 24 days documented in single-virus infections or their controls (Garza-Hernández et al. 2013). In a greenhouse experiment, after confining one fungus-exposed male (FEM) and one unexposed male (UM) with 20 female mosquitoes, the females marked by the FEM were double (seven) that those marked (three) by the UM. Moreover, the conidial load attached to one FEM averaged 50,000/ml, in contrast to 10,000/ml and 5,000/ml estimated for the first and fifth female mated by the same FEM, respectively, and that died in six days. This indicated that a single FEM is capable of infecting and killing up to five female mosquitoes (Garza-Hernandez et al. 2015).

The aforementioned information was used as a baseline to carry out a trial to test whether wild females marked by FEM were more abundant than those marked by UM using human-landing collections, after releasing 4,800 FEM in 15 treated households and 4,800 UM in 15 control households over the course of eight weeks at a rate of 40 males/household/week. It was found that in treated households, the rates of recaptured FEM and marked females were 0.050 (243/4,800) and 0.147 (29/197), respectively, which were 2.5 and 2.3 times higher than 0.020 (96/4,800) and 0.060 (22/365) of recaptured UM and marked females in the control households (Reyes-Villanueva et al. 2021). Therefore, if FEM are 2.5 times more prone than UM to swarm on humans and if marked/infected females succumb in 4-9 days by fungal infections acquired from FEM (Reyes-Villanueva et al. 2011, Garza-Hernandez et al. 2015), we tested the hypothesis inferred from the dataset reported elsewhere (Reyes-

Villanueva et al. 2021) that marked females as the response variable (count) must be more highly correlated with total females, marked males, and wild males in treated households than in control households. We did not explore this topic previously (Reyes-Villanueva et al. 2021) because it needs to be analyzed and published separately. The information is relevant to understanding the impact that *M. anisopliae* may exert upon *Ae. aegypti* populations disseminated from the release of males carrying fungal conidia in dengue hyper-endemic localities. Thus, the aim of this study was to examine the dataset and develop a generalized linear model (GLM), such as the Poisson or negative binomial distribution, suitable to predict marked females collected per week in 15 households treated with FEM and in the other 15 households where UM were released.

MATERIALS AND METHODS

Trial design

The survey was done in a neighborhood of Reynosa, México, a sister city of McAllen, Texas whose geography and climate were already described elsewhere (Rodriguez-Perez et al. 2021). The 30 experimental households are scattered across an area occupied by about 120 households, separated by a 200 m wide arid land, in two groups of approximately 60 households each. A two-arm design was used with 15 households randomly chosen from about 60 per arm; the northern side was designated as the treated arm, while the southern side was the control. The 200 m arid land served as a barrier to obstruct the interchange of mosquitoes between treatments. Experimental households were randomized only the first week and then were examined throughout the survey. Total females, marked females, marked males, and wild males were documented by human-landing captures done by a team (two volunteers: bait and collector, 20 min/household) from the 30 households per week, where captured mosquitoes were rapidly released after being counted. However, about three weeks prior to the survey, all mosquitoes were eliminated from experimental and non-experimental households by indoor spraying with deltamethrin 0.25 mg, delivering approximately 2.4 liters/household (in 3 min) and by removing all water-containers from backyards. A week later, human-landing counts were initiated in experimental households and the trial commenced the week in which at least one mosquito was collected in one experimental household. This permitted working with complete cohorts of *Ae. aegypti* during September and October, 2015, the months in which the highest vector densities occur in northeastern México (Salas-Luevano and Reyes-Villanueva 1994). Further methodological details are provided elsewhere (Reyes-Villanueva et al. (2021).

M. anisopliae culture and *Ae. aegypti* colony handling

The Ma-CBG-2 strain of *M. anisopliae* was cultured on potato-dextrose-agar in plates and incubated at 25° C for 20 d in the dark until sporulation. Conidia production was estimated by a mixture of 0.5% Tween-20 and 0.5% Triton-X in 0.85% saline solution, while conidial suspension centrifuged at 3,500 rpm for 10 min was diluted to $1.6x10^8$ conidia/ml estimated by hemocytometer counts. Lastly, 5 to 7 ml of the final suspension was applied to 8 cm diameter filter papers (2.5 mm pore) to produce $5.96x10^7$ dry conidia/cm^2 as reported earlier (Reyes-Villanueva et al. 2011).

The released male mosquitoes were four to six-day-old, unmated, sugar-fed *Ae. aegypti* taken from a colony established in 2006 with larvae from Monterrey, México and reared according to Reyes-Villanueva et al. (2011). Larvae were reared using 200 larvae/liter of deionized water in an enamel pan, pupae were confined in a screened cage, and females were blood-fed on the arm of the same volunteer. Afterwards, twenty virgin males were confined with either a fungal-treated or clean filter for 24 h in a chamber formed by two half-Petri plates, then transferred to a 1 liter meshed-cardboard cup for resting for 3 h. They were then marked with 0.5 g of yellow or red dust expelled through a bulb duster (Garza-Hernandez et al. 2015). Overall, 40 FEM in treated (20 red and 20 yellow) and 40 UM in control (20 red and 20 yellow) were released weekly per household (reds: released at living room, yellows: released at entrance) in 15 households for eight weeks, with a total of 4,800 FEM and 4,800 UM released in treated and control households, respectively (Reyes-Villanueva et al. 2021).

Statistical analysis

Counts of total females (TOF), marked females (MKF), marked males (MKM), and wild males (WM) collected in 15 households/week were documented in a dataset for a period of eight weeks. As MKF were wild females marked by red and yellow dust transferred from the forty FEM released weekly per household in treated and control sites, they comprised the response variable to be modeled. Prior to modeling, the ratio of the conditional variance to the mean (VMR) was calculated. This relationship was estimated for MKF in both treatment and control to determine the basic dispersion type. Overall, data were over-dispersed if VMR > 1, equi-dispersed if VMR = 1, and under-dispersed if VMR < 1 (McCullagh and Nelder 1989, Cameron and Triveldi 1990, Kruppa and Hothorn 2021). After modelling, the Pearson χ^2 and residual deviance statistics as dispersion parameters were computed for MKF (RD) per fitted model. The *RD* statistic is defined as twice the difference between the log-likelihood of the fitted model and the saturated model in which a perfect fit occurs with n parameters (Favero et al. 2021).

In the Poisson–negative binomial fit, the Pearson χ^2 or *RD* is transformed to a ratio by dividing each by their degrees of freedom (*df*), then the square root of each is computed giving the "scaled Pearson χ^2 ratio" ($\sqrt{\text{Pearson } \chi 2/df}$) or the "scaled deviance ratio" ($\sqrt{RD/df}$) and both are known as the dispersion parameter phi (ϕ) (SAS 1999). It is worth noticing that there are no exact limits on the dispersion parameter values to distinguish over-dispersion from equi-dispersion (Payne et al. 2018). It has been pointed out that a "strong" over-dispersion exists if the Pearson χ^2 ratio is equal or greater than 2 (Cameron and Triveldi 1990), by which we identified a "slight" over-dispersion when the Pearson χ^2 ratio, deviance ratio, or ϕ, were between 1 and 2, while an under-dispersed

Table 1. Goodness-of-fit statistics for two generalized Poisson regression models to predict marked *Aedes aegypti* female (Y) collected in 15 households/week through eight weeks in two groups of 15 households each. In treated, 40 *Metarhizium anisopliae*-exposed, dust-marked males were released/household/week; in controls, 40 unexposed, dust-marked males were released/household/week. In treated groups, the best fitted model comprised total females, marked males, and the total females*marked males' interaction as predictors. In controls, marked males was single predictor.

Treatment	Model parameter	Estimate (standard error)	Wald χ^2 ($p > \chi^2$)	Scaled Pearson[1] χ^2 /*df*[2]	Deviance of null model (*DN*)	Deviance of fitted model (*DF*)	Pseudo R^2 measure (1 - *DF*/ *DN*)	Pearson χ^2 for observed (O) *vs.* predicted (P) Y; (*df*, $p > 0.05$ for H_o: O = P) [3]
Treated				0.89	22.4916	1.0262	95%	0.92 (6, 0.92)
	Intercept	-2.2497 (0.89)	7.01 (0.0008)					
	Total females β value	0.0891 (0.01)	37.52 (0.0001)					
	Marked males β value	0.0811 (0.02)	10.96 0.0001					
	Total females*marked males β value	-0.0020 (0.0001)	20.66 (0.0001)					
Control				1.01	9.7206	5.2418	46%	5.33 (6, 0.50)
	Intercept	2.37 (0.64)	13.17 (0.001)					
	Marked males β value	-0.1235 (0.06)	4.09 (0.04)					

[1] The scale parameter was estimated by the square root of Pearson c^2/df. Goodness-of-fit shown by the ratio c^2/df was ~ 1.
[2] *df* = degrees of freedom.
[3] In both treatments, H_o was accepted as O and P marked females were equal significantly as P were predicted by the Poisson regression model ($c^2 > 0.05$)

model was recognized if these ratios were less than 1 (Kruppa and Hothorn 2021).

Moreover, the null model deviance (*DN*) and the fitted model deviance (*DF*) were also computed. The former estimates how well MKF is predicted by an intercept-based model in relation to a perfect model, while the later also evaluates how well MKF is forecasted but by a full model (intercept plus predictors) relative to a perfect model (Hastie 1987). With both *DN* and *DF* the "pseudo-R^2 measure" was computed to compare the best fitted model in treated against the one found in control households; the pseudo-R^2 measure is a ratio based on the difference between the "lack of fit" of each model with respect to the perfect model (Cameron and Windmeijer 1996, Mittlböck 2002). It is important to clarify that the pseudo-R^2 measure is not the Pearson R^2 coefficient of the least square method, but a ratio of deviances whose value also goes from 0 to 1, with 1 indicating a perfect fit where predicted/observed data match and predictor number is *n* (Cameron and Triveldi 1998, Khun and Johnson 2013). According to Heinzl and Mittlböck (2003), the pseudo-R^2 equation has the following form:

$$R^2 = 1 - (DF/DN)$$

where:
DF = deviance of the fitted model
DN = deviance of the null model

The goodness-of-fit (GOF) of MKF as response variable (Y) in 14 multivariate regression models (seven/treatment) to the standard Poisson (equi-dispersion), generalized Poisson (slight over-dispersion/under-dispersion), and negative binomial distribution model was explored. Each fitted model comprised TOF, MKM, and WM as predictors X_1, X_2, and X_3 and the examined models included the additive and those additive-interactive variables.

After finding the best fitted model to a GLM per treatment according to the values of Pearson χ^2 ratio, *RD* ratio is φ, within each model, the Wald χ^2 tests for coefficients significance (Ho: $\beta = 0$) plus p-value of the χ^2 linked to each coefficient β, the pseudo-R^2 measure (%), and the χ^2 test for the Poisson (Ho: model = Poisson vs Ha: model ≠ Poisson) are shown in Table 1. The weekly variation of the mean of MKF, the 95% asymmetrical confidence interval per week, and outliers are displayed in Figure 1. All statistical analyses were performed by using proc genmod with SAS software, version 8.

RESULTS

Overall, 1,083 mosquitoes were documented through the entire trial distributed in treated (560) and control (523) households as follows: 562 total females (197 and 365), 51 marked females (29 and 22), 339 marked males (243 and 96), and 131 wild males (91 and 40). In treated households, total females (TF) were reduced by 46% while marked females (MKF), marked males (MKM), and wild males (WM) increased by 24%, 60%, and 56%, respectively in comparison to those found in control households. The statistical comparison of these groups was similar to what has been reported by Reyes-Villanueva et al. (2021); data are also shown here.

Prior to modelling, the response variable MKF in treated households showed a strong over-dispersion, with a ratio of the conditional variance to the mean (VMR) of 5 (variance = 15.69, mean= 3.62 ± 1.40) in comparison to the slightly over-dispersed VMR ratio of 1.4 (variance = 3.98, mean=2.75 ± 0.70) observed in control households. After computing, the GOF of MKF in the seven regression models per treatment, we observed that the values of Pearson χ^2 ratio and *RD* ratio were very similar in the same regression model, so to simplify the results only the Pearson χ^2 ratio was used as dispersion parameter to compare fitted models. Table 1 shows all the GOF statistics, demonstrating that only two (one per treatment) out of 14 fitted regressions, exhibited a good fit, not to the standard (equi-dispersed) Poisson but to the generalized Poisson model, in which the scaled Pearson χ^2 or Pearson χ^2 ratio exhibited values close to one. The generalized Poisson is for examining slightly over-dispersed or under-dispersed data, in which, the standard Poisson variance = mean relationship is transformed to a variance = mean (ϕ), where the multiplicative effect of ϕ tends to keep equi-dispersion, without changing model parameters and estimators, but making the confidence limits more conservative (SAS 1999). In treated households, the best fitted model was multivariate: μ = exp (intercept = -2.2497) + exp (0.0891 total females) + exp (0.0811 marked males) + exp (-0.0020 total females*marked males), with a Pearson χ^2 ratio of 0.8988 about 0.90 (nearly equi-dispersion) and Poisson acceptance test with a $\chi^2 = 0.92$, $df = 4$, and P= 0.92. Thus, in control households, the best fitted model had just one predictor: μ = exp (intercept = 2.3707) + exp (-0.1235 marked males), showing a scaled Pearson χ^2 ratio of 1.01 (equi-dispersion) and a $\chi^2 = 5.33$, $df = 6$, and P = 0.50 for the Poisson acceptance test. Furthermore, the pseudo-R^2 measure computed in both treatments indicated that the best fitted model was found in treated households with a 95% lack-of-fit, exhibiting a better performance than the 46% lack-of-fit showed by the best model fitted in control households.

Lastly, Figure 1 depicts the expected weekly mean (μ) of MKF computed by the best fitted model plus the 95% asymmetrical confidence interval lines according to the generalized Poisson model; there was just one outlier in the treated group compared to two in the control, proving that the generalized Poisson in the treated group was more suitable to predict marked female *Ae. aegypti*.

DISCUSSION

This is the first report concerning the prediction of host-seeking, marked *Ae. aegypti* females in households where *M. anisopliae*-exposed males were released weekly for eight weeks at a dengue endemic site. It is also the first time that a generalized Poisson model is used to forecast host-seeking females of the dengue vector in the field. Though the best fitted model in treated households exhibited a slight under-dispersion (Pearson χ^2 ratio = 0.90), it was the best to predict

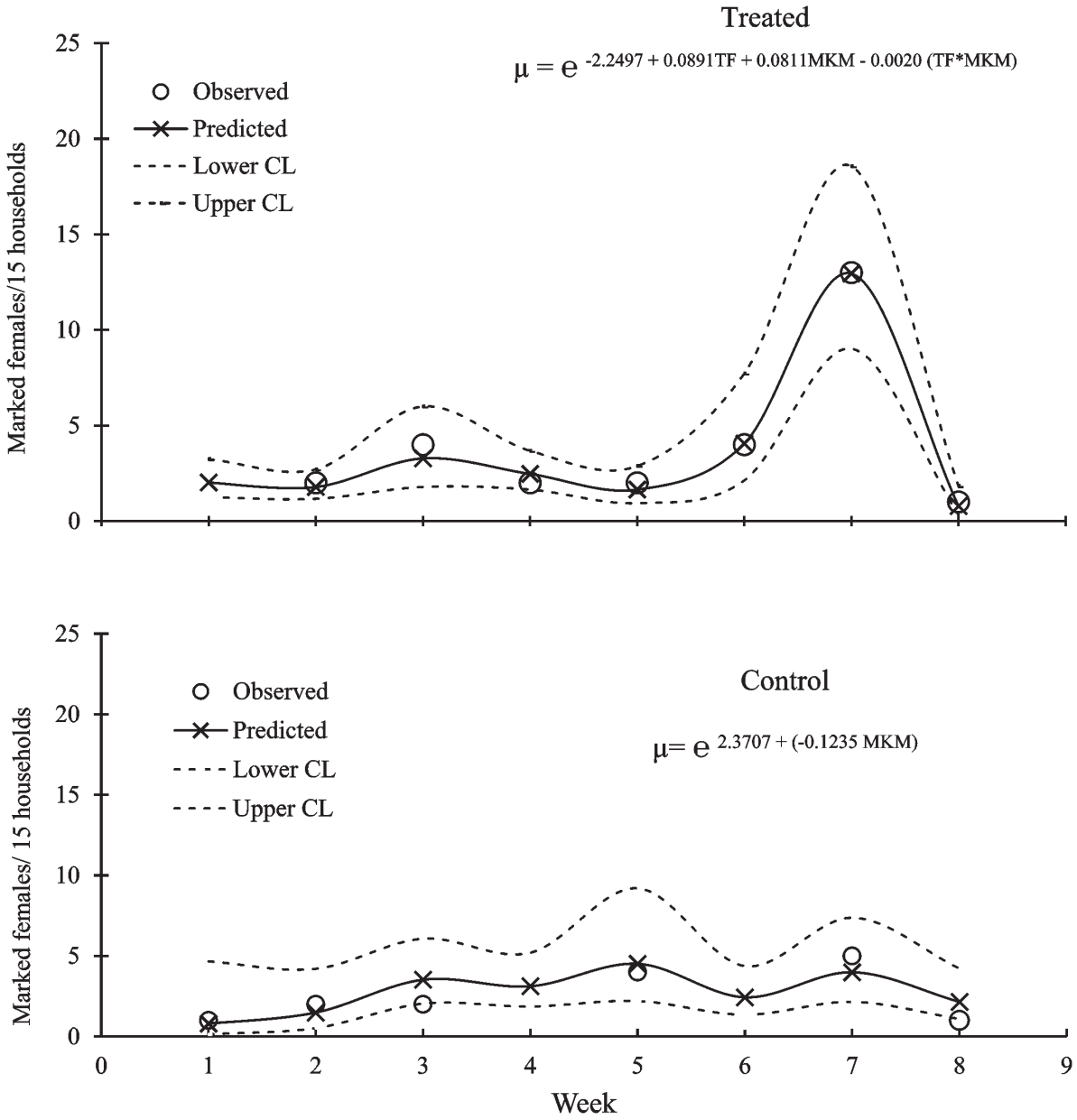

Figure 1. Mean of predicted (X) and number of observed (**O**) marked *Aedes aegypti* females, lower (Lower CL) and upper (Upper CL) asymmetrical 95% confidence limits plus outliers (●) documented for eight weeks in two groups (treated and control) of households of 15 each. In the treated group, 40 *Metarhizium anisopliae*-exposed, dusted males were released per household/week, while in controls, 40 unexposed, dusted males were released per household/week. In the treated group, females were predicted by a generalized Poisson regression model with total females (TF), marked males (MKM), and the TF*MKF interaction as strong ($\chi^2 < 0.05$) predictors, whereas in controls, MKM was the single predictor. The model is shown at top right of each plot.

MKF according to the pseudo-R^2 measure (95% lack-of-fit) and the χ^2 test for Poisson acceptance (χ^2 = 0.92, df = 6, P = 0.92).

It is difficult to discuss results without similar reports. Here, the monitoring of *Ae. aegypti* was done by human-landing captures in which the mosquitoes were counted when they landed after swarming over a volunteer (Salas-Luevano and Reyes-Villanueva 1994); thus, this survey provides information about the *Ae. aegypti* swarm size but not about mosquito density in treated and control households. We did not carry out an alternative sampling/trapping method to estimate mosquitoes, therefore, we cannot explain the 46% reduction of total females in treated households, as in our collections of corpses to determine the sporulated fungal strain spread by FEM we only found one positive corpse. Moreover, the presence of outliers was due perhaps to a small sample size (n = 8), where each sample unit comprised the total mosquitoes counted in 15 households/treatment/week and the study lasted only eight weeks. However, the surveyed time was similar (about six weekly releases in 44 days) to that reported by Trewin et al. (2021) in Australia, where from 1,228 to 1,250 rhodamine B-marked males/week were released to estimate dispersal by MKF in traps. We analyzed the open-access data and found a good fit to the generalized Poisson model (scale = Pearson). Reported and computed (in the present work) MKF in six samplings were 22 (13), 28 (27), 9 (13), 13 (14), 13 (14), and 13 (14), respectively; the GOF statistics for the model were acceptable except for the intercept coefficient (P = 0.61), while the released males were the single predictor in the model.

A better explanation of the effects of predictors upon the number of MKF comes by interpreting the signs and exponentiated coefficients to see the antilog (e^{β}). The negative intercept (-2.2497) in the multivariate model from treated households means that the expected number (mean) of MKF was < 0, or more precisely it was the decimal 0.1054 ($e^{-2.2497}$), which is about zero. In contrast, the positive intercept (2.3707) of the univariate model in control households suggests the existence of 10.70 MKF (= $e^{2.3707}$) in conditions of zero marked males (SAS 1995). In the treated model, the reasoning within this study context is that there were not MKF because all allegedly died due to the *M. anisopliae* infection, in comparison to that of controls where MKF survived because UM were free of fungus. Nevertheless, if the slope of a predictor X is significant ($\beta_x > 0$), that would mean that for each 1-unit increase in X_1, the expected number of Y would be Y (e^{β} - 1) assuming that other predictors are holding constant. Thus, in the treated model, the slope as exponent (e^{β}) of the three predictors are (antilog) 1.0931, 1.0844, and 0.9980; after one is subtracted from them the resultant percentages are therefore 9%, 8%, and -0.002%, respectively. We can thus conclude that in households where *M. anisopliae*-exposed males were released, the increase of one TF or one marked male would correspond to an increase of 9% or 8%, respectively, in MKF, while the effect of the interaction of both is still negative and was an irrelevant predictor to the model. However, in control households. the increase of one UM would decrease the number of MKF by 12%.

Finally, the Poisson model found here needs to be tested further using large scale studies involving at least 50 households treated with FEM and another 50 treated with UM, before concluding that it predicts females infected by the males previously exposed to *M. anisopliae* and released in households. It should then be possible to use this model to indirectly estimate the *Ae. aegypti* MKF from the FEM releases and evaluate the model paired to the *Ae. aegypti* male release programs available today.

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