Supplementary Material

§2. Comparison to Data and Exploration of Alternative Model Assumptions

Comparison to Data

We compare model output to data published in Wise de Valdez et al. (2011), whose laboratory experiments follow the same protocol outlined in our manuscript. We emphasize that although we can use our model to simulate the experimental design of these experiments, there are a number of parameter values for these experiments that are unknown (e.g. daily survival in the cage environment, average daily fecundity, and distribution of emergence times). Before using the model to simulate potential experiments, it is critical to obtain this information. For a given parameter set, we see that the model captures the general dynamics seen in the laboratory experiments by Wise de Valdez et al. (Figure S1a). Their data, however, exhibit more variability than the model, which reflects sources of variation that are not considered in our model, such as overdispersion in the daily egg production. The extinction times of two of the three populations in laboratory cages do fall within the distribution of extinction times predicted by the model for the parameters used here (Figure S1b).
Figure S1. (a) Dynamics of Eggs from data collected in laboratory experiments by Wise de Valdez et al. (2011) compared to model output. Blue, Red, and Green lines represent data. The solid black line represents the median behavior of 1000 simulations and the black dashed lines represent the 5th and 95th percentiles of 1000 simulations. (b) Histogram of extinction times from 1000 simulations with the same parameters used in (a). Note that the extinction times observed in Wise de Valdez et al. (2011) were 10, 15, and 20 weeks post release. For these simulations, $N = 200$, $r = 10$, $c = 0$, $s_f = 0.85$, $s_m = 0.72$, and $\lambda$ varies with female age, $a$, according to the function $\lambda(a) = -0.1543a + 35.694$ for $a = 6, \ldots, 35$ and $\lambda(a) = 0$ for $a < 6$ and $a > 35$, which is obtained by fitting the model to weekly egg counts obtained by Wise de Valdez et al.

Exploration of Alternative Model Assumptions

In the main text, we present results from a variety of simulations that are aimed at addressing the design of cage experiments. Throughout, we make simplifying assumptions regarding survival, mating behavior, and fecundity; however, the model is designed in such a way that these assumptions can be relaxed in order to study scenarios that incorporate more biological detail. In this section of the supplementary material, we present results from simulations in which more detailed descriptions of relevant biological processes (i.e., survival, mating, and fecundity) are considered. The aim here is to understand potential impacts of biological complexities not considered in the main text that could have played a role in the outcomes of previous experiments. Further, we consider a variant of the current experimental protocol that would allow for delay periods between input of wild-type and FK individuals, a change that might ease the daily work load of personnel responsible for rearing and managing the mosquito populations. We revisit immigration of wild-type individuals by studying the immigration of adults, and we study how correlation between extinction time and reductions in the wild-type adult female population depend upon the time at which reduction is measured.
Survival

In the main text, we assume constant daily adult survival. Here, we consider age-dependent survival based on data presented in Figure 1 of Styer et al. (2007). We consider four different release ratios, $r = 0.1, r = 1, r = 10, r = 100$. To begin studying the effects of age-dependent survival, we take average daily survival probabilities for females and males that lead to average lifespans equivalent to those calculated in Styer et al. (2007), about 54 days for females and 30 days for males. We obtain $s_f = .9818$ and $s_m = .9679$. We note that the survival curve when the mortality rate is constant must have a longer tail in order to obtain an average lifespan equivalent to that that results from mortality that is age-dependent. We compare cage experiments when survival is assumed to be age-dependent with those in which survival is held constant (Figure S2). We find that, for all four release ratios considered, extinction times are greater when constant survival is considered. While the average lifespan is the same for both cases, the long-tailed survival curve resulting from constant daily survival leads to a higher percentage of mosquitoes with long lifespans. For both age-dependent and constant survival, the extinction times predicted here are far greater than those predicted in the main text because the average lifespans observed in the Styer et al. (2007) data are much longer than those seen in the field and assumed in the main text.

![Figure S2](image)

**Figure S2.** Extinction time for two different survival scenarios with experiments conducted with four different release ratios. (a) Survival is age-dependent with daily survival probability obtained from data presented in Styer et al. (2007). (b) Survival is constant with respect to age. Circles are mean extinction time and error bars represent mean ± standard deviation. For these simulations, $N = 200$, $\lambda = 10$, and $c = 0$.

In order to make a better comparison with the main text, we scale the age-dependent survival probabilities from Styer et al. (2007) to obtain lifespans more similar to those resulting from the constant probabilities of survival used throughout the main text and the remainder of the supplementary material. For the constant probability of survival, we take $s_f = .9$ and $s_m = .79$. We take this value of $s_m$ (rather than taking $s_m = .72$ as in the main text) to match the ratio of the male and female average daily survival obtained from
Styer et al. (2007). With constant daily survival given by these values, average lifespan is about 10 days for females and 4.76 days for males, which reflects average lifespans observed in the field. We multiply the age-dependent survival probabilities in Styer et al. (2007) by a constant \((k_f = 0.9021\) for females and \(k_m = 0.7928\) for males) in order to equate the average lifespan with that resulting from constant daily survival. Results are presented in Figure S3 below.

![Figure S3](image)

**Figure S3.** Extinction time for two different survival scenarios with experiments conducted with four different release ratios. (a) Survival is age-dependent with adjusted daily survival obtained from data presented in Styer et al. (2007). (b) Survival is constant with respect to age. Circles are mean extinction time and error bars represent mean ± standard deviation. For these simulations, \(N = 200\), \(\lambda = 10\), and \(c = 0\).

For all four release ratios, we find that the mean and variation of extinction times are slightly higher when daily survival is taken to be constant across age classes. The maximum difference in mean extinction time is about 10 days when \(r = 0.1\). This indicates that the simplifying assumption of constant daily survival does not have a significant impact on the extinction times that the model predicts when lifespans are short.

We note, however, that average lifespan can have significant impacts on the extinction times predicted by the model. For instance, if we use the age-specific daily survival probabilities directly obtained from Styer et al. (2007), we find that extinction times are approximately double those in which daily survival is adjusted for a shorter lifespan (refer to Figure S2). This result underscores the need for an accurate assessment of the environment-specific life history characteristics of the pest species being studied in cage experiments. Knowledge of the average lifespan and survival curve of the species will improve the predictive ability of the model.

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Mating Behavior

Here, we look at the impact on extinction time of polyandrous mating of females. Females are considered to be polyandrous if they mate on two or more occasions in their lifetime. We consider only the case in which a female mates twice. We assume that each mating occurs on separate days and that the female mates with two different males. First, we assume that sperm from each mating has an equal probability of fertilizing an egg, and we consider matings that occur 7, 14, 21, 28, and 35 days apart under four different release ratios. Results are presented in Figure S4.

**Figure S4.** Extinction time for treatment cages for experiments conducted with four different release ratios. Females are assumed to be polyandrous and sperm from each of the females’ two matings have an equal probability of fertilizing eggs. (a) $r = 0.1$, (b) $r = 1$, (c) $r = 10$, and (d) $r = 100$. Circles are mean extinction time and error bars represent mean $\pm$ standard deviation. The label ‘SM’ denotes the case in which only a single mating occurs. In these simulations, $N = 200$, $\lambda = 10$, $c = 0$, $s_f = 0.9$, and $s_m = 0.72$.

Overall, we find that there is little difference in mean extinction time between simulations in which females only mate once and those in which two matings occur. For larger release ratios, the mean time to extinction decreases slightly when matings occur 7 and 14 days apart, but for longer periods between mating, the mean extinction time is similar to the cases when females only mate once.
The overall lack of differences in extinction times for different periods between the two matings could be due to the low impact that the two matings have on the population over the duration of the experiment. While females who mate twice should be more likely to mate with homozygous FK males when they mate a second time than when they first mate, this increase in likelihood will be most significant just after FK males have been initially released. Wild-type frequency decrease most rapidly during the early weeks of releases and this rate of decrease gradually slows until the wild-type population is extinct. The reduced impact of the longer periods between matings is also a result of mortality. That is, fewer females survive to mate a second time when the period between matings is longer.

![Figure S5. Extinction time for treatment cages for experiments conducted with four different release ratios.](image)

Females are assumed to be polyandrous and sperm from each of the females’ two matings have a different probability of fertilizing eggs, which is determined by the degree of polyandry. (a) \( r = 0.1 \), (b) \( r = 1 \), (c) \( r = 10 \), and (d) \( r = 100 \). Circles are mean extinction time and error bars represent mean ± standard deviation. In these simulations, \( N = 200 \), \( \lambda = 10 \), \( c = 0 \), \( s_f = 0.9 \), and \( s_m = 0.72 \).

The contribution of polyandrous mating seems rather minimal when sperm are used equally from each mating; however, the probability that sperm from one mating fertilize eggs need not be the same as that of the other mating. We consider experiments in which offspring are more likely to receive sperm from one mating than the other. We define the
**degree of polyandry** to be ratio of sperm from the second mating to the total sperm available to fertilize eggs. For example, if the degree of polyandry is 0, none of the sperm used in fertilization are from the second mating; if the degree of polyandry is 1, all of the sperm used in fertilization are from the second mating. We study the effects of different degrees of polyandry on extinction time when the two matings occur 7 days apart. Results are shown in Figure S5.

In general, extinction time decreases as the degree of polyandry increases from 0 to 1. This decrease is greater when release ratios are smaller. For all four release ratios, when all of the offspring have fathers from the second mating (i.e., the degree of polyandry is 1), the mean extinction time is the lowest. With increased degree of polyandry, a greater portion of the offspring have fathers from the second mating, which results in faster decline of the wild-type population.
Fecundity

Next, we consider age-dependent fecundity. Rather than assuming females of all ages produce the same number of offspring on average, we allow for different fecundity values based on the age of the female. We use age-dependent fecundity values taken from data presented in Figure 3 of Styer et al. (2007). In the results presented here for the constant fecundity, we take $\lambda = 8$. We take this average from the data by calculating the average of the expected number of offspring a female of a certain age has given that she survives to that age. That is, if $l_x$ is the probability that a female lives to age $x$ and $m_x$ is the expected number of total offspring of a female of age $x$, we take $\lambda = \left\lceil \frac{1}{L} \sum_{x=1}^{n} l_x m_x \right\rceil$. Here, $L = \sum_{a'} l_{a'}$ is the average lifespan of a reproductive female (i.e. the average number of days a female is able to lay eggs) and $\lceil \cdot \rceil$ denotes the ceiling function. Results are shown in Figure S6.

![Figure S6](image_url)

**Figure S6.** Extinction time for treatment cages for two different fecundity scenarios when experiments are conducted with four different release ratios. (a) Age-dependent fecundity is taken from Styer et al. (2007) and (b) Fecundity is constant across age classes. Circles are mean extinction time and error bars represent mean ± standard deviation. In these simulations, $N = 200$, $c = 0$, $s_f = 0.9$, and $s_m = 0.72$.

For all four release ratios considered, the mean extinction time and variation in extinction time is slightly lower when fecundity is constant across age classes. This is likely the case because, based on the data obtained from Styer et. al (2007), young females have more offspring on average than older females. The average value used for constant daily survival is averaged across all age classes and is thus influenced by the low number of eggs laid by older females. The increase in the number of offspring results in larger wild-type populations that must be suppressed.
Input Delay

In some cases, the logistics of carrying out the experiment may make it desirable to separate the releases of larvae hatched from eggs laid in the cage and those of FK mosquitoes by delaying the release of FK mosquitoes by several days. However, doing so could lead to female mosquitoes mating with wild-type males before FK males are introduced. Here, we examine the impact of FK introduction delays under the four release ratios. We consider delays of between 1 and 6 days and compare to the case in which there is no delay between introductions. See Figure S7 for the results.

![Figure S7](image)

**Figure S7.** Extinction time for treatment cages when there is a delay between the input of homozygous FK males and non-homozygous FK individuals with experiments conducted with a range of four different release ratios. (a) $r = 0.1$, (b) $r = 1$, (c) $r = 10$, and (d) $r = 100$. Circles are mean extinction time and error bars represent mean ± standard deviation. For these simulations, $N = 200$, $c = 0$, $s_f = 0.9$, and $s_m = 0.72$.

We find that under all release ratios except $r = 100$, mean extinction time increases to a maximum for delays mid-week, then decreases as the delays increase. This trend is a result of the overall proximity of the releases of FK individuals to the release of the other individuals. That is, input delays of 1 day or 6 days have less impact than delays of 3-4 days because the release of FK males in the former two cases occur within a day of the input of other individuals. When FK males are released mid-week, they are less likely to mate with
those individuals that are released at the beginning of the week or at the beginning of the next week, which leads to a slower decrease in the population. Input delays of 2-3 days seem to have a greater impact on experiments conducted with smaller release ratios. For $r = 0.1$, extinction occurs earlier on average with delays of 5-6 days. This likely occurs because FK pupae that are released at the end of the week emerge and are ready to mate at the same time as the female larvae that are released earlier in the week. This is less noticeable for larger release ratios. For $r = 100$, the increase in extinction time is almost linearly increasing with delay time. In this case, after the first few releases, the population is inundated with FK males. The delay in releases does not have a great impact on the population because the ratio of FK males to wild-type males is always very large. The delays then lead to extinction times that correspond to a scenario in which all releases are shifted by the number of days of delay. For example, if the mean extinction time with no delays is 100 days, the mean extinction time for a two-day delay would be roughly 102 days.
Immigration

In the main text, we considered an experimental design in which the effects of juvenile immigration can be studied in a cage experiment. Here, we extend this study further to consider the effects of adult immigration. We study the introduction of newly emerged, unmated adults and three day old, mated adult females and mating males by introducing additional adults of the age cohort under consideration. To make fair comparisons with larval immigration, we account for mortality of a cohort between larval and adult stages. We do this by selecting the number of migrating mosquitoes from a binomial distribution with parameters $N_l$ and $p_x$, where $N_l$ is the number of migrating larvae considered in the main text (10, 20, 30, 40, or 50) and $p_x$ is the probability that larvae survive to adulthood and live to age $x$. For newly emerged adults, $p_0 = (1 - 0.2318)$ and for three-day old adults, $p_3 = (1 - 0.2318)(0.5s_m^3 + 0.5s_f^3)$. Because immigrants are constantly being introduced into the population, we must observe population reduction as defined in the main text rather than extinction time. Results are presented in Figure S8.

![Figure S8. Percent wild-type adult females remaining 14 weeks post-release when wild-type immigrants are introduced to the cages every week. Experiments are conducted with four different release ratios. (a),(e) $r = 0.1$; (b),(f) $r = 1$; (c),(g) $r = 10$; and (d),(h) $r = 100$. (a)-(d) Immigrants are newly emerged, unmated adults. (e)-(h) Immigrants are three days old and females have mated before immigration. Circles are mean extinction time and error bars represent mean ± standard deviation. For these simulations, $N = 200$, $c = 0$, $\lambda = 10$, $s_f = 0.9$, and $s_m = 0.72$.](image)

As in the case of juvenile immigrants, we see a gradual increase in the mean and variance of percentage of the wild-type adult female population remaining after 14 weeks as the number of immigrants increases (Figure 9, main text). The only exception is, again, $r = 0.1$, in which the variance decreases as the number introduced increases. In general, immigration of the three day old adults seems to inhibit population reduction only slightly less than newly emerged adults, as the mean and variance of the percentage of the wild-type adult
female population remaining is slightly lower in all cases when adult immigrants are three
days old than when they are newly emerged. In many cases, however, the difference is less
than one percent. In all cases, immigration of juveniles results in a higher percentage of
females remaining than does immigration of adults.

Our model output indicates that if already-mated adults enter a population, the adult
female population will be maintained at a low level, because all offspring of the mated
immigrants will be wild-type. This differs slightly from the result when immigrants have
not yet mated. In the latter case, once the target population is sufficiently suppressed,
the immigrant females would have a high probability of mating with FK males and the
population would consist of only the immigrants themselves.
An Alternative Measure for Assessment of Population Reduction

We analyze the results generated by the model for several different scenarios that arise from the combinations of fitness costs and release ratios (i.e., results presented in Figure 6) to assess the relationship between extinction time and wild-type adult female population reductions after a given period of time. We obtain the coefficient of determination $R^2$ from a linear regression of extinction time against percentage of the wild-type adult female population remaining after a given number of weeks (here, $n = 1000$ simulations is the total sample size). We present here four different scenarios that result from considering two release ratios ($r = 1$ and $r = 10$) and two fitness costs ($c = 0$ and $c = 0.6$).

![Figure S9](image)

**Figure S9.** The correlation between cage extinction time and population reduction after a given time period. Values of the coefficient of variation $R^2$ are plotted for each week post-release for (a) $r = 1$ and (b) $r = 10$. Circles represent simulations run with $c = 0$ and squares $c = 0.6$. For these simulations, $N = 200$, $\lambda = 10$, $s_m = 0.72$, $s_f = 0.9$.

Figure S9 shows that the value of $R^2$ can be very different depending upon the time at which population reduction is observed. In general, population reduction as measured by observing the percentage of wild-type adult females remaining 15-30 weeks post-release is a better predictor of extinction time than observing reduction earlier than 15 or later than 30 weeks when $r = 1$. When $r = 10$, that window is earlier and more narrow, around 10-20 weeks. These so-called windows of higher correlation correspond to periods of time after reductions in population size have begun but before a large number of extinctions have occurred. In fact, the peaks of the curves in Figure S9 are partially a result of extinctions beginning to occur. That is, as $R^2$ increases to the maxima seen here, extinctions are beginning to occur before the time of observation of population reduction. The strength of predictions that can be made based on observing population reduction then decreases rapidly as the time post release gets longer. Therefore, observing population reduction before the times associated with these peaks would be best for using the model to make predictions.
References
