MODELLING THE EFFECTS OF STRESSORS AND TREATMENT IN A HONEYBEE COLONY:

THOMAS LUIK

A THESIS SUBMITTED TO
THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

GRADUATE PROGRAM IN MATHEMATICS AND STATISTICS
YORK UNIVERSITY
TORONTO, ONTARIO

DECEMBER 2023

Abstract

Honeybees are agriculturally important through their pollination work and production of honey. They are also vulnerable to the accumulation of stressors, which cause drastic colony losses. It is important to understand the effect of stress on a hive so that the cost of testing, treatment, and rest can be evaluated. We have developed a model of honeybee stress and simulated scenarios of testing and treatment regiments. The model tracks importation of stressors through foraging, and quantifies their effect on bee stress. The model is used to determine appropriate times for testing and treatment before, during, and after pollination jobs, and it is used to determine resting periods needed between pollination jobs, depending on testing and treatment use, to minimize the probability of bee loss and maximize profit for a beekeeper. Ultimately, the model will be used to inform testing and treatment strategies that will increase economic profitability for the beekeeping industry, and the agricultural sector as a whole.

Acknowledgements

This thesis would never have been possible without the help and cooperation of the following people:

To my supervisors, Dr. Jane Heffernan and Dr. Matt Betti for their mathematical and computational guidance.

To Dr. Amro Zayed and the entirety of BeeCSI for their melittological knowledge and guidance.

And to my family for their continuous support throughout my time writing this thesis.

Contents

Abstract ii

Acknowledgements iii

Contents iv

List of Tables vi

List of Figures vii

1 Introduction 8

1.1 The Western Honeybee 10

1.2 Honeybees and Pollination 12

1.3 Stressors 13

1.4 Testing 15

1.5 Treatment 15

1.6 The Khoury Model 16

1.7 Other Models of Honeybee Colonies 18

1.8 Scope 19

2 Methods 19

2.1 Model 20

2.2 Proof of Existence and Boundedness 23

2.3 Numerical Simulations and Sensitivity Analysis 24

3 Results 28

3.1 Steady states 28

3.2 Numerical Simulations 29

4 Discussion 43

4.1 Conclusions 43

4.2 Future research 45

References 47

List of Tables

1 The structure of the honeybee population in one colony. 11
2 Stressors that can cause honeybee decline [4]. 13
3 Simulation Scenarios. "All" refers to the fact that all pollination job lengths are shown in Section (A) in each figure for the scenarios. (X days) refers to the pollination job length shown in section (B) in each figure for the scenarios. 26
4 Parameter values for various levels of stress. 27

List of Figures

1 Flow diagram of the Khoury model. 17

2 The flow diagram for our model. There are different levels of stress experienced by bees. The cleaning coefficients, γ_n, are set to zero before treatment is applied. When treatment is active, these coefficients are then set to the treatment parameters. . . . 20

3 Scenario 1 from Table 3. 31

4 Scenario 2 from Table 3. 34

5 Scenario 3 from Table 3. 36

6 Scenario 4 from Table 3. 37

7 Scenario 5 from Table 3. 39

8 Scenario 6 from Table 3. 40

9 Scenario 7 from Table 3. 41

10 Scenario 8 from Table 3. 51

Modelling the Effects of Stressors and Treatment in a Honeybee Colony

Thomas Luik

December 2023

1 Introduction

Honeybees are the most agriculturally important species of animal in Canada due to their ability to pollinate fruit through sheer numbers [5]. Being a eusocial species allows the honeybees to send out enough foragers to pollinate many crops, but the large numbers make it difficult for beekeepers to diagnose the various stressors (such as harmful chemicals, parasitic organisms and harsh weather conditions) afflicting colonies before they become a larger problem. Testing for stressors can be difficult, depending on stressor type and frequency of beekeeper observation [4]. Additionally, in some cases, a test can take up to 2 months to diagnose a stressor [4] by which time the affected colony could drastically decline or experience colony collapse. Beekeepers of declining colonies usually point to the death of a queen as the cause of the collapse of a colony [4]. However, the death of a queen could reflect colony stress level, related to dysfunction of the hive and forager bees in a colony due to stress; if detected early, this dysfunction could be mediated. Treatment of the whole colony is important because it is the workers who feed the queen and her brood (the eggs she has laid that have hatched into larvae) and maintain a colony so that she can spend all her time laying eggs. If workers become infected or poisoned, then the queen can also be infected or poisoned, or starve from a lack of effective workers.

The honeybee research community has identified a need to better understand colony stress and determine methods for testing and treatment that can be accessible and manageable for the beekeeping industry [4]. In particular, the research community has been asked to determine an effective testing and treatment strategy for colony stress, including identifying appropriate testing times after a pollination job, time to communication of result, time of treatment initiation, and length of treatment needed to ensure that a stressed colony will be healthy enough to survive the winter.

In this thesis, we develop a mathematical model of honeybee stress, testing and treatment. The model, which is an extension of a model by Khoury et al. (The Khoury model) [14], is used to track the level of stress (or stressors) in the honeybee colony over time. These stressors can be imported during a pollination job, and decay from cleaning behavior, natural degradation, and treatment. Using this model, we study the outcomes of different testing and treatment scenarios on honeybee colonies that exit pollination jobs with different levels of stress. We tackle questions such as: what is the effect of identifying and treating a colony earlier? Is there an effect on recovery time? For different rates of stressor accumulation and decay, can we identify a ”sweet spot” for testing and treatment that will allow bee colonies to thrive and complete pollination jobs for the agricultural sector?

In the sections that follow, we introduce the biology of honeybees and their colony. We then provide an introduction to stressors that affect honeybee survival, followed by a brief overview of currently used treatments. Next, we introduce the Khoury model [14] that we extend for the current work. Finally, we present and analyze our extended model and offer possibilities for future research.

1.1 The Western Honeybee

The western honeybee, *Apis mellifera*, is the key subject of interest for this thesis as our work is in collaboration with bee researchers in Canada that study this organism.

The western honeybee is an insect that contributes to the economy by producing honey, and more importantly, pollinating crops to increase their yield [9]. In Canada in 2016, the contribution of honey to the economy was $253,500,000 CAD [1] and the value added to crops was $2,570,583,000 CAD [1] .

The western honeybee is eusocial, which is a social structure marked by: the presence of reproductive and non-reproductives females; the longevity of the reproductive female generally exceeding that of her non-reproductive offspring; siblings assisting in brood rearing; feeding the brood at regular intervals; division of labor between reproductive and non-reproductive castes; hive and food storage construction; swarming being incorporated into the mating process; the colony being perennial; and communication among colony members [19].

Honeybees are hymenopterans [18], and therefore have haploid-diploid sex determination where males have a single set of chromosomes (haploid) and females have two sets of chromosomes (diploid) [6]. As a consequence, females are related to each other by a factor of 0.75 instead of a typical 0.5, and thus have a stronger genetic incentive to help in the reproduction of siblings [18]. This system allows honeybees to work as a collective, contributing to brood rearing, and the stockpiling of large quantities of pollen, nectar, and wax, to foster an environment suited towards reproduction [20].

In a honeybee colony, there are several different life stages present and dif-

Life stage	Function	Description
Egg	To be laid and then hatch into larvae	The eggs laid by the queen that may hatch into a drone or a female.
Larva	To be fed and then pupate	The larvae remain inside their cells and are fed by workers. The kind of food given to female larvae determines if they become a queen or a worker.
Pupa	To eclose into a queen, a worker, or a drone	Larvae are capped for pupation.
Queen	To lay eggs	There is a single queen for a colony that lays all of the eggs.
Hive	To care for the brood, clean the hive, and learn to become a forager.	The bees that spend their time doing tasks and working within the hive. They eventually become foragers.
Forager	To bring provisions back to the hive and slow the process of hive bees maturing into foragers.	The final stage of life for a worker bee.
Drone	Produce sperm and mate with queens	The male honeybees.

Table 1: The structure of the honeybee population in one colony.

ferent types of workers. These are summarized in Table 1.

Concerns about thermoregulation, the ability of an organism to regulate body temperature, factors into colony survival during winter [10]. To regulate body temperature at lower external temperatures, honeybees will generate heat using their shivering flight muscles [10]. It is generally accepted that the absolute minimum amount of bees needed to survive the winter due to thermoregulation concerns is approximately 15,000 bees [7]. As clarification, this threshold is not one that ensures survival, but ensures that the colony has the possibility of survival. Colonies that would be under this threshold by winter time would have to consider being merged together [7].

We note that for the current work, we consider stress affecting the honeybee colony. Given the large numbers of hive and forager bees, like Khoury et al. [14], we focus our work on hive and forager bees. However, we do include the effects on colony dynamics of reproduction (activities including the queen and the brood) and maturation of hive bees into foragers.

1.2 Honeybees and Pollination

Due to the ability of a colony to visit several thousand flowers over a given season, honeybee colonies are able to acquire large quantities of nectar stored as honey, as well as ensure the fertilization of several thousand fruits [9]. Flowers may need multiple visits for a bee to fertilize all of the ovaries on a given flower [24]. If complete fertilization does not not happen, then the fruit produced from the flower will be malformed or non-existent, thus decreasing the yield [24].

Given their ability to pollinate and thus increase the quality of plant yield, honeybee colonies from beekeeping operations are often contracted out for use in commercial pollination jobs in the agricultural sector [19]. In Canada, honeybees therefore contribute to the overall agricultural sector and are key to agricultural success [2].

The global stock of honeybees is growing at a slower rate than the agricultural demand for the crops that they pollinate [3]. It is therefore important to maximize the probability of existing colony survival, and increase colony health, so that more pollination jobs can be feasible for a colony. In this thesis, our mathematical modelling study works to understand the effects of testing and treatment post-pollination job. The purpose of this study is to highlight how earlier testing and treatment can increase colony recovery from stress accumulated during the pollination job. The ensuing benefits ensure that: (1) more pollination jobs can be scheduled, and (2) a honeybee colony reaches the critical population threshold so that overwinter survival is maximized.

Stressor	Type
Clothianidin	pesticide
Thiamethoxam	pesticide
American foulbrood	disease
Chalkbrood	disease
Nosema ceranae	disease
Israeli acute paralysis virus	disease
Varroa destructor	parasite
Amitraz	acaricide
Formic acid	acaricide
Oxalic acid	acaricide
Oxytetracycline	antibiotic
Spinetoram	non-systemic insecticide
Spirotetramat	non-systemic insecticide
Chlorantraniliprole	systemic insecticide
Flupyradifurone	systemic insecticide
Sulfoxaflor	systemic insecticide
Glyphosate	herbicide
Fludioxonil	fungicide
Metconazole	fungicide
Boscalid	fungicide
Fluopyram	fungicide
Pyraclostrobin	fungicide
Pyrimethanil	fungicide

Table 2: Stressors that can cause honeybee decline [4].

1.3 Stressors

There are many different stressors of honeybees. These include poisons, parasites, and bacterial and viral infections. Table 2 lists some examples.

Pesticides are problematic for bees. Poisons such as pesticides and insecticides are simply chemicals that exist and interact with an organism's biology in a negative way. For example, neonicotinoids kill bees by binding to the bees' nicotinic acetylcholine receptors and shutting down their central nervous system [11]. Bees could be in pesticide and insecticide contaminated areas without the beekeeper knowing. Additionally, pesticides that normally do not affect bees can break down into deadlier components or mix with other seemingly benign pesticides to become deadly. For example, fungicides such as boscalid can interact with insecticides such as chlorantraniliprole to cause increased death to honeybees [23]. Poisoning is typically identified when the colony abruptly decreases in number, and the natural rhythm of roles being filled is disrupted, collapsing the colony [19]. Poisons can be generalized as a category to include all accumulated objects that can be considered harmful to bees.

Parasites are incredibly destructive to colonies. Ectoparasites, such as the *Varroa destructor* mite, are parasites that live on the outside of a host bee. They feed off of the bee and are vectors of honeybee diseases, such as twisted wing virus. By feeding on the brood, the virus is spread to the larvae which then develop malformed wings [19]. Endoparasites, such as *Nosema* microsporidia, are parasites that live inside a host bee [19]. *Nosema* inflames the midgut, which both starves the bees and causes them to have diarrhea and defecate feces laden with *Nosema* spores throughout the colony [19].

Bacterial and viral infections are serious to bee colonies. Some countries require burning colonies infected with certain stressors of this category, such as American foulbrood [19].

For the purposes of the present study, we have focused a generalized stressor. We assume that the stressor can be imported into the hive. We do not explicitly model the growth of the stressor on it's own in the hive. Thus, the general stressor is likened more towards a poison rather than a parasite that can multiply on it's own. Extensions of the model to include more explicit stressor multiplication functions is a course for future work and is beyond the scope of the present study [19].

Further, while many different types of stressors have been identified, specific model parameters associated with cleaning, death rate, importation rate, etc., have not been estimated. Therefore, a sensitivity analysis will be conducted to explore different stressor scenarios related to these parameters, as well as different testing and treatment rates over all classes or categories of bee stress within a colony. Our model examines stressors that accumulate in the colony from importation on forager bees from the field to the hive. Exposure to stressors will increase the stress level of the individual bees in the colony such that there can be multiple individuals with different stress levels within the entire colony.

1.4 Testing

There is no current common practice for beekeepers to test their colonies before or after a pollination job or at other times of the year [4]. Therefore, there exists the risk that testing occurs only after the collapse of a colony is imminent and nothing can be done to prevent or remediate it. Quantification of the benefits of testing and treatment can help optimize the decision making process of a beekeeper/beekeeping organization to assign colonies to pollination jobs. However, the costs of testing and treatment must not outweigh the benefits of these activities that work to ensure honeybee health and the success of the overall agricultural sector.

1.5 Treatment

There are many different actions that a beekeeper can take to treat a stressed colony, including combining weakened colonies, requeening, destroying contaminated hive materials, feeding the colony to maintain numbers, and drug treatment [19]. Treatments can also include activities that work to degrade pesticides in the colony, or rid the colony of parasites [19]. In the current study, treatment will be generalized as a transition of bees to a lower category of stress which may best reflect a drug treatment being applied to the colony.

1.6 The Khoury Model

Our model is an extension of the Khoury model [14] to include the effects of a generalized stressor to the bee colony. The Khoury model examines the general functionings of a colony without considering the effect of any stressor.

The Khoury model studies the life cycle of worker bees (hive and forager bees) from performing tasks within the hive to foraging outside. It demonstrates the following assumptions: (1) the forager bees regulate the maturation of hive bees into forager bees; (2) the survival of the brood is a function of both worker castes; (3) the survival of hive bees is high due to the protected nature of the hive to the point of their mortality being negligible.

The Khoury model considers the female worker population only, since the male reproductive caste does not affect the work done to ensure survival of the colony. The queen is also not considered explicitly, as events concerning her are usually discrete, and it would be more useful to assume that she is replaced if she dies. Finally, the Khoury model does not include equations for brood populations and assumes that the brood survival depends solely on the laying rate of the queen and the activity of the worker bees.

A flow diagram of the Khoury model is presented in Figure 1. The model equations are:

$$\begin{aligned} \tfrac{dH}{dt} &= L\tfrac{H+F}{H+F+w} - (\alpha - \sigma\tfrac{F}{H+F})H \\ \tfrac{dF}{dt} &= (\alpha - \sigma\tfrac{F}{H+F})H - \mu F\,. \end{aligned} \tag{1}$$

In the Khoury model, it is assumed that there are only two castes of worker bees of significance to the colony where a caste refers to the role of a bee in the colony. H is the number of bees performing work within the hive and F is the number of bees who forage for resources. It is assumed that there is no overlap between the two roles. A hill function based on total number of bees determines percent survival of the eggs laid by the queen reaching adulthood. Parameter

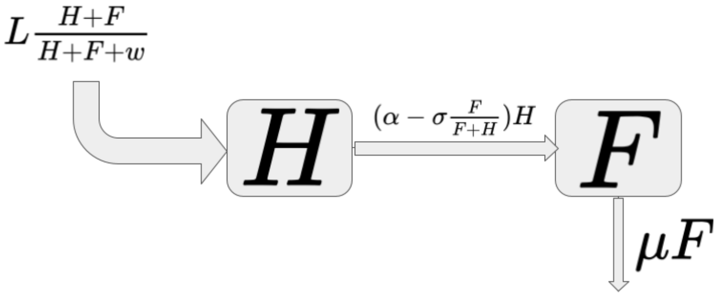


Figure 1: Flow diagram of the Khoury model.

w represents the amount of bees needed to achieve 50 percent survival of the brood until their eclosion, the emergence of an insect from a pupa. Pupae eclose into hive bees and perform various tasks around the colony. We note that the queen is relegated to a parameter in the eclosion function $E(H,F) = L\frac{H+F}{H+F+w}$, namely, L, the laying rate of eggs. In the timeframe of the Khoury model, it is assumed that the queen is always present. This is most likely due to the average lifespan of a queen being two to five years and the average worker lifespan being between 15 and 38 days [19]. It is assumed that all of the brood become hive bees. Hive bee death rates are considered negligible due to being in the protected environment of the colony.

The forager class recruits from the hive bee class and the forager class dies at rate μ. The recruitment function $R(H,F)$ is assumed to have the following structure, $R(H,F) = (\alpha - \sigma\frac{F}{H+F})H$, where α is the maximum recruitment rate for hive bees into the forager caste, which theoretically occurs when there are no foragers present in the colony. $\sigma\frac{F}{H+F}$ represents how the presence of foragers reduces the rate of recruitment. It is assumed that social inhibition is directly proportional to the total number of foragers as a percentage of the colony. The Khoury model assumes: $\alpha = 0.25$ per day because hive bees typically becoming foragers 4 days after eclosion in the absence of foragers [14]; $\sigma = 0.75$ per day because this rate of inhibition would only result in a reversion of foragers to hive bees only if more than one third of the colony are foragers; the daily laying rate is $L = 2000$ eggs laid per day; and $w = 27,000$ individual bees.

The Khoury model, depicted in Figure 1, examines the fragility of a bee colony due to changes in the death rate (reflecting the effects of pesticides, parasites, etc.). Their major finding is that the death rate of foragers increasing beyond a certain threshold causes the collapse of a colony.

H and F bees accumulate stress which could affect their death rates. In the current work, we extend the Khoury model to include different levels of stress in H and F bees so that we can explore this potential increase in lethality. We depict our model in Figure 2 and describe it in Section Methods.

1.7 Other Models of Honeybee Colonies

There are other models of honeybee colonies that we have not considered because they include factors that are beyond the scope of this study. For example, there are different models for which the laying rate of the queen in relation to the amount of workers is a generalized hill function of $\frac{(H+F)^i}{(H+F)^i+K^i}$ [17], instead of the basic hill function presented in the Khoury model [14], where the function is $\frac{H+F}{H+F+w}$. A consequence of having $i > 1$ is that there is the possibility of two positive equilibrium points [17]. We did not add this in our model as the value of i is generally unknown and we are focusing of the well-being of a colony with one hive size.

As well, there are models in which worker bees are reduced to one significant caste, such as the model by Magal et al., which considers only a population of foragers [15]. Other models add other stressors as factors. Spores of parasites such as *Nosema* can be thought of as building up and infecting members of the colony. [16]. Further, Khoury et al. has expanded on their own previous model by including food as a factor [22]. Finally, there are other models which explicitly take a brood population into account [13]. However, these models use delay differential equations which are outside of the scope of this thesis.

We have used the Khoury model as a basis [14] because it captures the fragility of a honeybee colony through lack of social inhibition as well as the straightforward increase in death rates. That is to say, it presents a possible death spiral mechanic independent of outright death.

1.8 Scope

In this thesis, we are concerned with how a honeybee colony is affected by a generalized stressor, which includes any combination of stressors listed in Table 2. We extend the Khoury model to include different levels of stress. We also include different significant rates of the stressor importation, pollination job length, time to testing, time to treatment, and treatment length. The goal is to identify a testing and treatment strategy that can maximize pollination jobs and probability of colony survival that is also feasible for the beekeeper. We note that our model presented here deals only with ordinary differential equations, and is therefore deterministic. It does not take into account stochastic effects.

2 Methods

In this section, we introduce our model of honeybee stress and treatment. We also introduce tools that we use to analyze the model.

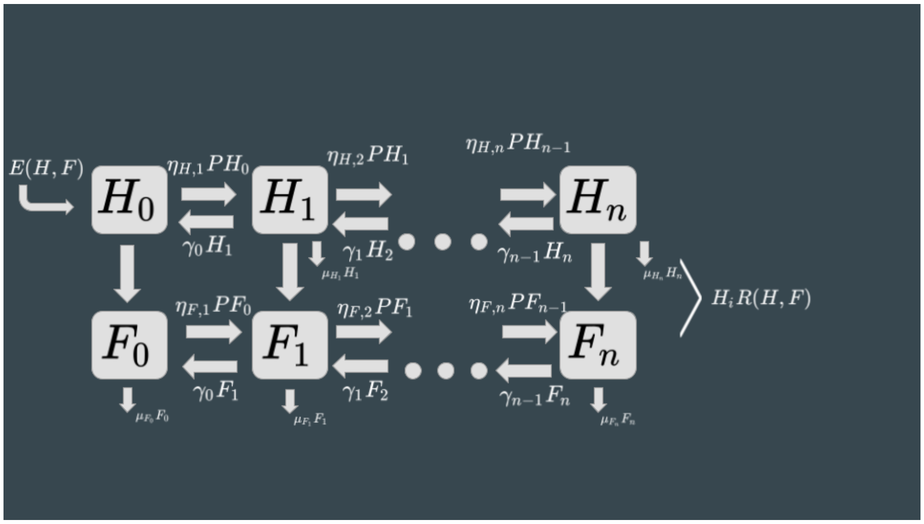


Figure 2: The flow diagram for our model. There are different levels of stress experienced by bees. The cleaning coefficients, γ_n, are set to zero before treatment is applied. When treatment is active, these coefficients are then set to the treatment parameters.

2.1 Model

We extend the Khoury model, shown above in Figure 1, to include different levels of stress in the hive H and forager F bees. There are two major assumptions:

1. H and F bees can become increasingly stressed through contact with the stressor in the colony, but they can also experience a decrease in stress. The increase and decrease of stress are modeled using acquirement and decay functions whereby the rate of increase or decrease in stress depends on the corresponding rate for the stress level of the bee. It is assumed that these functions can take different shapes for the H and F bees. The degradation function can be modified through intervention by the beekeeper.

2. The stressor is imported into the colony by the forager bees. The amount of stressor imported into the colony therefore depends on the number of

forager bees in the colony and their activity, but it also depends on the amount of stressor, (P), being considered. The impacts of higher and lower rates of stressor importation, (a), are therefore considered. The total level of stress in the hive total is related to the total number of stressed hive and forager bees.

A flow diagram of our model is shown in Figure 2. The model equations are:

$$
\begin{aligned}
\frac{dH_0}{dt} &= L\frac{\sum_{n=0}^{S} k_n H_n + \sum_{n=0}^{S} k_n F_n}{\sum_{n=0}^{S} H_n + \sum_{n=0}^{S} F_n + w} - \left(\alpha - \sigma \frac{\sum_{n=0}^{S} F_n}{\sum_{n=0}^{S} H_n + \sum_{n=0}^{S} F_n}\right) H_0 - \eta_0 P H_0 + \gamma_{1,H} H_1 \\
\frac{dH_n}{dt} &= -\left(\alpha - \sigma \frac{\sum_{n=0}^{S} F_n}{\sum_{n=0}^{S} H_n + \sum_{n=0}^{S} F_n}\right) H_n - \eta_n P H_n + \eta_{n-1} P H_{n-1} + \gamma_{n+1,H} H_{n+1} - \mu_{n,H} H_n - \gamma_{n,H} H_n \\
\frac{dH_S}{dt} &= -\left(\alpha - \sigma \frac{\sum_{n=0}^{S} F_n}{\sum_{n=0}^{S} H_n + \sum_{n=0}^{S} F_n}\right) H_S + \eta_{S-1} P H_{S-1} - \mu_{S,H} H_S - \gamma_{S,H} H_S \\
\frac{dF_0}{dt} &= \left(\alpha - \sigma \frac{\sum_{n=0}^{S} F_n}{\sum_{n=0}^{S} H_n + \sum_{n=0}^{S} F_n}\right) H_0 - \eta_0 P F_0 + \gamma_{1,F} F_1 - \mu_{0,F} F_0 \\
\frac{dF_n}{dt} &= \left(\alpha - \sigma \frac{\sum_{n=0}^{S} F_n}{\sum_{n=0}^{S} H_n + \sum_{n=0}^{S} F_n}\right) H_n - \eta_n P F_n + \eta_{n-1} P F_{n-1} + \gamma_{n+1,F} F_{n+1} - \mu_{n,F} F_n - \gamma_{n,F} F_n \\
\frac{dF_S}{dt} &= \left(\alpha - \sigma \frac{\sum_{n=0}^{S} F_n}{\sum_{n=0}^{S} H_n + \sum_{n=0}^{S} F_n}\right) H_S + \eta_{S-1} P H_{S-1} - \mu_{S,F} F_S - \gamma_{S,F} F_S \\
\frac{dP}{dt} &= a \sum_{n=0}^{S} a_n F_n - c \frac{P}{P + K_{max}} \sum_{n=0}^{S} c_n H_n - dP
\end{aligned}
\tag{2}
$$

Here, we track changes in H and F bees over time. We consider $S+1$ levels of stress. That is, $0 \leq n \leq S$, where n is the stress level of H_n and F_n bees, zero denotes no stress, and S denotes the maximum level of stress on a scale of 0 to S. For the purpose of this work, we consider $S = 9$.

Similar to the Khoury model, we include eclosion and recruitment functions. It is assumed that new H bees only enter the zero-stress level, H_0, but that H_n bees with stress level n are recruited to F_n, the forger bees with the same stress level. Note that we assume that parameters w, σ and α from the Khoury model are independent of any stress level. This is assumed so that, in the absence of stress, or in the presence of very little stress, our model does not differ from that discussed in the Khoury model [14]. Additionally, it is not known how these parameters are affected by stress in the colony, so they cannot be quantified. Finally, as the current work is focused on treatment and testing of the H and F bees, and not on interventions that are directly related to eclosion or recruitment, we have kept these parameters constant.

Parameters η_n and γ_n denote the rate at which a bee leaves a category of stress, either by the rate at which a bee interacts with a stressor and becomes more stressed, or how fast a bee recovers to a lower level of stress, respectively. η_n is the rate at which after interaction with the stressor P, the bee with stress level n goes up a category of stress to $n + 1$. This is assumed to be through the law of mass action [12], making the term $\eta_n P$. γ_n is assumed to be a decay rate of stress such that a bee with stress level n goes down to stress level $n - 1$ which may happen naturally, but it is more likely to occur with treatment over time. As it is not known how quickly stress can accumulate or decay, we vary the stress transition arrays in a sensitivity analysis.

In terms of mortality rates, we assume that the bees with no stress have the same mortality rates as those in the Khoury model, which is 0 for hive bees and 0.14 bees per day for foragers [14]. We assume that higher stress levels in hive and forager bees will increase their mortality rates, due to oxidative stress.

Stressors, represented by P, are assumed to be any single stressor or combination of stressors listed in Table 2. We consider a general stressor here, since estimates of the model parameters related to the stressors in Table 2 do not exist. The stressor amount in the colony is determined by the behaviour of the hive and forager bees. It is assumed that the foragers import stressors and that the importation rate, a_n, depends on the foragers' stress level n. The stressor can also be cleaned by hive bees. It is also assumed that the cleaning activity depends on the stress level of the H bees. We assume that both the rate of stressor importation and the rate of natural enzymatic cleaning are diminished by stress. These assumptions are represented by the respective coefficients of a_n and c_n, such that $a_n, c_n \leq 1$. For the simulations in this model, $a_n = c_n = 2^{-n}$.

Due to the enzymatic nature of the breakdown, it is assumed that the bees can only break down P as a hill function of itself with a maximum saturation of decay, K_{max}[8]. Finally, we assume that the stressor can decay naturally in the hive with rate d. For example, pesticides, which can be brought into the hive by foragers, can decay both by natural exponential decay and by bees processing it through digestion. Note that in certain scenarios, the value of this enzymatic digestion is zero. This is because there are cases in which the substance cannot be broken down or is broken down into another substance that is still considered stressful to the bee.

Various treatment rates are included in the model allowing for stressors to decay slowly or quickly depending on the stress level of the bees. We consider many different sets of treatment parameters, as the efficacy of treatments on bees has not been comprehensively modeled. There are scenarios in which certain categories of stressed bees cannot be treated. We include a set of treatment parameters in our analysis that applies treatment only to low-stressed bees. Conversely, we also include a set of treatment parameters that focuses on only the highly stressed bees, as these may be easier to identify through behaviour change by beekeepers, and thus could be treated directly. This scenario of only applying treatment to highly stressed bee can also account for situations in which there are potentially other stressors within the colony that do not get treated, and so the bees can only return to a middle level of stress and can go no lower.

2.2 Proof of Existence and Boundedness

The following proof uses results taken from [17], which proved the existence and boundedness of the solutions of the base model [17]. When $(H_0, H_1, H_2, H_3, H_4, H_5, H_6, H_7, H_8, H_9, F_0, F_1, F_2, F$ are all zero, the derivative equals $(\frac{L}{w}, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)$.

The system is differentiable, due to automatically satisfying the Lipschitz condition, a unique solution exists.

Now, let

$C' = (\sum_{n=0}^{S} H_n + \sum_{n=0}^{S} F_n)' = L\frac{H+F}{H+F+w} - (\sum_{n=0}^{S} \mu_{n,H} H_n + \sum_{n=0}^{S} \mu_{n,H} F_n)$

and $\mu_{min} = min\{\mu_{n,H}, \mu_{n,F}\} > 0$.

Then C is bounded by Q, where $Q'(t) = L - (2S+1)\mu_{min} Q(t)$.

Solving this, we obtain $Q(t) = Q(0)e^{-(2S+1)\mu_{min} t} + \frac{L}{2S+1}$. For our model, where $S = 9$ and $\mu_{min} = 0.01$, the equation is $Q(t) = Q(0)e^{-0.19t} + \frac{L}{19}$. Because of this, our system is bounded.

2.3 Numerical Simulations and Sensitivity Analysis

We note that while some analytical results related to our model will be presented, numerical simulations are used to track the H and F bees over time. To run our model, we assign initial conditions which represent the Stressor Free Equilibrium, although rounded to integer values:

$$(H_0(0), H_1(0), H_3(0), H_4(0), H_5(0), H_6(0), H_7(0), H_8(0), H_9(0)) = (19642, 0, 0, 0, 0, 0, 0, 0, 0, 0)$$

$$(F_0(0), F_1(0), F_3(0), F_4(0), F_5(0), F_6(0), F_7(0), F_8(0), F_9(0)) = (7110, 0, 0, 0, 0, 0, 0, 0, 0, 0)$$

$$P(0) = 0$$

The Stressor Free Equilibrium represents the maximum population of bees in the colony, and the fraction residing in the H_0 and F_0 classes. The simulation shows the scenario in which a beekeeper takes a colony into a field in order to perform a pollination job. After a few days of letting the colony approach a maximal value, the bees are then taken to a field for a pollination job. This initiates nonzero values for the stressor importation parameter a_n, as it is assumed that the field has some stressor in it. The pollination job is run for N days where N is informed by a collaborator-led survey of beekeepers [4]. The stressor importation rate and stress accumulation functions in the H and F bees are manipulated to see their effects.

It is assumed that the beekeeper opens the hive for observation or testing M days after the end of the pollination job. To account for the time for a diagnosis, we assume that treatment can be initiated Q days after observation and testing. The treatment is provided to the hive and forager bees for T days. The end of the pollination job, the time in which treatment is started, and the treatment time are all manipulated in order to see their effects.

Various sets of treatment parameters are used for the F and H bees. These functions serve to: test if treatment has any effect at all; tell if treatments should focus on the hive or forager bees; and see how different functions affect the treatment of the bees to allow for another pollination job, or to reach the critical survival threshold for overwintering. Through a review of the literature, this amount was determined to be 15,000 bees [7].

Due to the variability of decay rates for stressors such as pesticide, we consider different rates of natural decay of the stressor in the hive. Additionally, we allow for degradation of the stressor in the hive through cleaning. The cleaning parameter, c, is set to either 0 or 0.0048.

Tables 3 and 4 list all of the possible values of the model parameters and functions that are used in our study. In order for the model to be informative, the parameters chosen for the time-based parameters were round numbers or common intervals, such as 0, 1, 2, or 5 days, one to two weeks, one to two months, or one year. These were used because they correspond to time intervals used in the BeeCSI survey of various beekeepers [4]. This study provides commonly accepted intervals for beekeepers. However, for treatment times, it is also assumed that such round numbers could affect effective treatment application

Parameter	Meaning	Value
N	Pollination job length	[7 15 30 60]
Q	Treatment start time	[1 7 14]
T	Treatment length	[0 1 2 5 7 14 365]
c	Cleaning parameter	[0 0.048]
a	Stressor importation rate	[0.0000048 0.000048 0.06]

Scenario		
1	Pollination job length	All (7 days)
	Treatment start time	1 day after end of pollination job
	Treatment length	All
	Cleaning parameter	0.048
	Stressor importation rate	0.000048
2	Pollination job length	All (15 days)
	Treatment start time	1 day after end of pollination job
	Treatment length	All
	Cleaning parameter	0.048
	Stressor importation rate	0.000048
3	Pollination job length	All (30 days)
	Treatment start time	1 day after end of pollination job
	Treatment length	All
	Cleaning parameter	0.048
	Stressor importation rate	0.000048
4	Pollination job length	All (60 days)
	Treatment start time	1 day after end of pollination job
	Treatment length	All
	Cleaning parameter	0.048
	Stressor importation rate	0.000048
5	Pollination job length	All (15 days)
	Treatment start time	7 days after end of pollination job
	Treatment length	All
	Cleaning parameter	0
	Stressor importation rate	0.000048
6	Pollination job length	All (30 days)
	Treatment start time	7 days after end of pollination job
	Treatment length	All
	Cleaning parameter	0.048
	Stressor importation rate	0.0000048
7	Pollination job length	All (60 days)
	Treatment start time	14 days after end of pollination job
	Treatment length	All
	Cleaning parameter	0
	Stressor importation rate	0.0000048
8	Pollination job length	All (7 days)
	Treatment start time	1 day after end of pollination job
	Treatment length	All
	Cleaning parameter	0
	Stressor importation rate	0.06

Table 3: Simulation Scenarios. ”All” refers to the fact that all pollination job lengths are shown in Section (A) in each figure for the scenarios. (X days) refers to the pollination job length shown in section (B) in each figure for the scenarios.

Parameter	Definition	Value	Reference
w	Amount of bees that guarantee at least half maximum eclosion rate	5000	Khoury et al. 2011
μ_{0F}	Death rate of unstressed foragers	0.14	Khoury et al. 2011
L	Laying rate	2000	Khoury et al. 2011
α	Maximum recruitment	0.25	Khoury et al. 2011
σ	Inhibition from recruitment by foragers	0.75	Khoury et al. 2011
η_n	Stress transition rate from stress level n to $n+1$	varies	assumed an increasing function - see in methods
γ_n	Recovery rate from stress level n to $n-1$	varies	assumed different functions such that the area under the γ curve is constant - see Table 4
a_i	Import rate of stressor to the hive by F bees in the nth level of stress	varies	assumed a decreasing function - see in methods
c_i	Cleaning rate provided by H bees in the nth level of stress	varies	assumed a decreasing function - see in methods
k_i	Rearing impediment for H and F bees in the nth level of stress	varies	assumed a decreasing function - see in methods
$\mu_{n,H}$	Death rate for H bees in the nth level of stress	varies	see in methods
$\mu_{n,F}$	Death rate for F bees in the nth level of stress	varies	see in methods

	Stress level									
Parameter	0	1	2	3	4	5	6	7	8	9
a_n	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.0020
c_n	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.0020
$\eta_{n,H}$	0.1	0.13	0.15	0.16	0.167	0.171	0.175	0.178	0.18	N/A
$\eta_{n,F}$	0.11	0.14	0.16	0.17	0.177	0.181	0.185	0.188	0.19	N/A
γ_n	[0 10]	[0 10]	[0 10]	[0 10]	[0 10]	[0 5 10]	[0 10]	[0 10]	[0 10]	[0 10]
$k_{n,H}$,$k_{n,F}$	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	0.0020

Table 4: Parameter values for various levels of stress.

by the beekeeper to the colony, i.e., adherence to providing treatment should be simple and easy to remember. The rationale behind this is similar to how pharmacies prescribe medicine to be taken in commonly recognized intervals in order to aid patient compliance.

We note that Scenario 6 from Table 3 presents a best case scenario due to the combined factors of low stressor importation and the ability of the bees to naturally dispose of the stressor. Scenario 8 presents a worst case scenario due to a high rate of stressor importation and no natural ability for the bees to dispose of the stressor.

3 Results

3.1 Steady states

The steady state equilibrium of Equation (1), which is also the disease-free model of Equation (2), is:

$$F_0 = \frac{L}{\mu} - w\frac{J}{J+1}, \qquad H_0 = \frac{F_0}{J}$$

where

$$J = \frac{1}{2}\left(\left(\frac{\alpha}{\mu} - \frac{\sigma}{\mu} - 1\right) + \sqrt{\left(\frac{\alpha}{\mu} - \frac{\sigma}{\mu} - 1\right)^2 + 4\frac{\alpha}{\mu}}\right)$$

which can be interpreted as the equilibrium ratio of the forager bees to the hive bees. The two populations rapidly establish this ratio [14].

The Jacobian at (H_0, F_0) is

$$J = \begin{bmatrix} L\frac{w}{(w+H_0(1+J))^2} - \alpha + \sigma\frac{J^2}{(1+J)^2} & L\frac{w}{(w+H_0(1+J))^2} + \frac{\sigma}{(1+J)^2} \\ \alpha - \sigma\frac{J^2}{(1+J)^2} & -\frac{\sigma}{(1+J)^2} - \mu \end{bmatrix}$$

Using the fact that $\alpha = \mu J + \sigma \frac{J}{J+1}$ [17] we can see that the determinant of J is positive when $\frac{Lw}{(w+H_0(1+J))^2} < \mu \frac{J}{(1+J)}$. As well, the trace of J is negative. Additionally, given that $H_0 = \frac{F_0}{J} = \frac{L}{J\mu} - w\frac{1}{J+1}$, we find that this inequality can be simplified to

$$\frac{L(J+1)}{Jw\mu} > 1$$

for the stability of the non-zero equilibrium. This condition can be interpreted in different ways biologically. Note that $\frac{L}{w}$ represents the eclosion rate without any assistance from the worker bees. This is modified by the death rate of the forager bees, μ. $\frac{J}{J+1}$ is the ratio of forager bees, F, to the rest of the colony at equilibrium. These results correspond to the Khoury model because our model, free of stressors, is at base the Khoury model. This was derived from the condition at equilibrium. Another interpretation could be the amount of eggs laid within the lifetime of a bee, $L(J+1)/J\mu$ and diminished by w.

3.2 Numerical Simulations

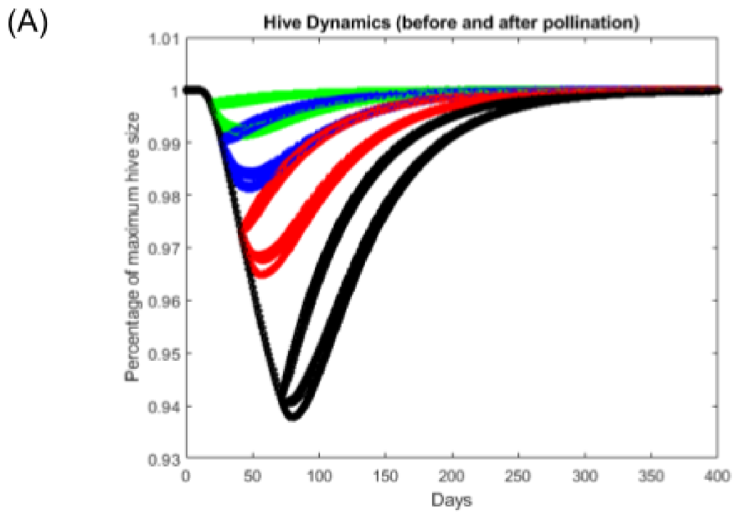
Numerical simulations are used to observe the accumulation and decay of stress in the colony, in H and F bees. In the following, we present results for the scenarios listed in Table 3. In Figures 3 to 10, we present:

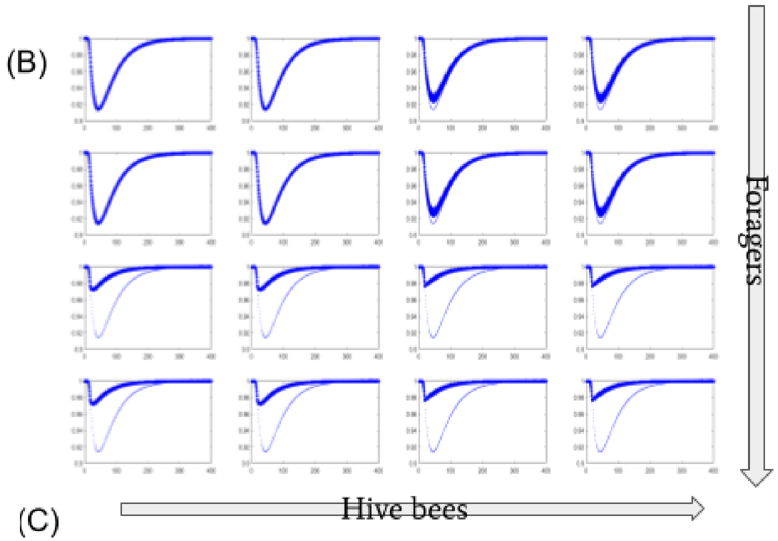
- Panel (A): Here, we show “All” pollination job lengths and “All” treatment lengths for all combinations of sets of treatment parameters for the H and F bees. The green scenarios are the outcome of a week-long pollination job (7-day), the blue scenarios are from a half-month-long pollination job (15-day), the red scenarios are from a month-long (30-day) pollination job, and the black scenarios are from a two-month-long (60-day) pollination job. All treatment lengths are shown but the colour does not vary. Panel (B) aids in determining what treatment the treatment length and combinations of sets of treatment parameters are for the H and F bees.

- Panel (B): Here, we show one scenario of the pollination job length (N days) as listed in Table 3, and include "All" treatment lengths. We also include the "no treatment" scenario (thin blue line). The progression along the horizontal axis as indicated by the arrow depicts the rates of effectiveness of the following treatment options on hive bees: no treatment; treatment of the most stressed hive bees; treatment of the least stressed bees; treatment of all hive bees; and extreme treatment of all hive bees. The progression down the vertical axis as indicated by the arrow shows the same treatment options but on forager bees: no treatment; treatment of the most stressed forager bees; treatment of the least stressed forager bees; treatment of all forager bees; and extreme treatment of all forager bees. The sub x-axes display the days over which the simulation takes place. The simulations run from 0 to 400 days. The sub y-axes display the fraction of the theoretical maximum colony size of the model.

- Panel (C): The statistics presented are the times in which certain percentages of the maximum colony size are reached. The table also shows what minimum size the colony reaches. Each column in the table corresponds to a subplot in Panel (B) as read from right-to-left; top-to-bottom. A "burn-in" of 10 days is added to the total as well as the number of days in the pollination job.

In Figure 3, we consider Scenario 1 from Table 3. Here, we allow for pollination jobs of 1 week, half a month, a month, and two months in length, corresponding to the green, blue, red and black colours in Panel (A). We then consider sixteen scenarios for sets of treatment parameters and seven scenarios for treatment length (see Table 3), where treatment is commenced 1 day after the end of the pollination job. In Panels (B) and (C), we consider the week-long pollination job scenario only (corresponding to the green curves in Panel (A)).





(C)

Min size (%)	91.40	91.40	92.71	92.71	91.40	91.40	92.71	92.71	97.25	97.25	97.66	97.66	97.25	97.25	97.66	97.66
Min time (day)	44	44	46	46	44	44	46	46	26	26	19	19	26	26	19	19
Time 95%	64	64	54	54	64	64	54	54	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Time 99%	150	150	141	141	150	150	141	141	79	79	63	63	79	79	63	63

Figure 3: Scenario 1 from Table 3. Here, we allow for pollination jobs of 1 week, half a month, a month, and two months in length, corresponding to the green, blue, red and black colours in Panel (A). We then consider sixteen scenarios for sets of treatment parameters and seven scenarios for treatment length (see Table 3), where treatment is commenced 1 day after the end of the pollination job. In Panels (B) and (C), we consider the week-long pollination job scenario only (corresponding to the green curves in Panel (A)), but include all treatment lengths. In Panel (B), we see that, intuitively, "no treatment" is the worst scenario (the thin blue line that reaches the minimum population value). However, we see that treatment will benefit the colony, reducing the recovery time of the colony back to maximum size. The largest reductions in recovery time correspond to treatments that preferentially treat forager bees F. The worst (red text) and best (green text) case scenarios for treatment are highlighted in the table presented in Panel (C). Overall, the best outcome corresponds to a minimum size of approximately 97%, which is reached at day 19, 9 days after the initiation of and 2 days after the end of the pollination job. We also observe that the colony reaches 95% and 99% of maximal colony size zero and 46 days after the end of the pollination job (corresponding to days 17 and 63 of the simulation). In some cases, the minimum size of a treated colony is greater than 95% (shown using N/A in Panel (C)).

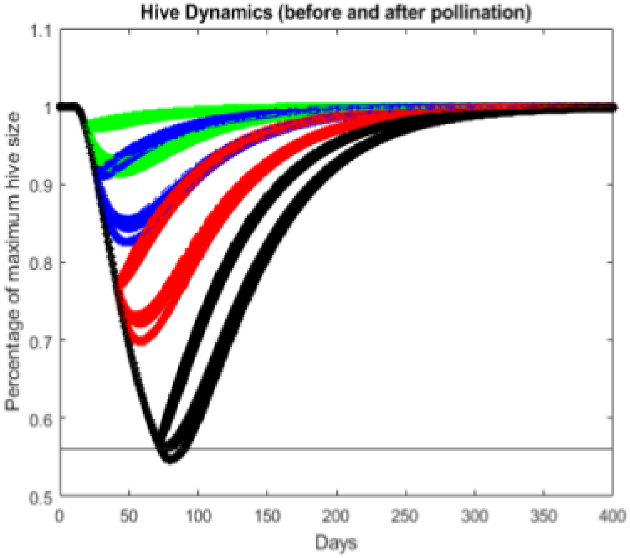
In Panel (B), we see that, intuitively, "no treatment" is the worst scenario (the thin blue line that reaches the minimum population value). However, we see that treatment will benefit the colony, reducing the recovery time of the colony back to maximum size. The largest reductions in recovery time correspond to treatments that preferentially treat forager bees F. The worst (red text) and best (green text) case scenarios for treatment are highlighted in the table presented in Panel (C). Overall, the best outcome corresponds to a minimum size of approximately 97%, which is reached at day 19, 9 days after the initiation of and 2 days after the end of the pollination job. We also observe that the colony reaches 95% and 99% of maximal colony size 0 and 46 days after the end of the pollination job (corresponding to days 17 and 63 of the simulation). In some cases, the minimum size of a treated colony is greater than 95% (shown using N/A in Panel (C)).

We note that Scenario 1 does not have a significant drop in any treatment outcomes. There does not seem to be an urgency in applying treatment in this scenario. However, the application of treatment is still effective in reducing the time until full recovery by approximately 3 months. Overall, as demonstrated by the difference as between subplots (Row 4, Column 1) and (Row 1, Column 4), there is a large difference in the outcome from treating all foragers (Row 4 Column 1) versus treating all hive bees (Row 1, Column 4), namely, that treatment of all foragers has a greater effect on the recovery of the entire colony than if only hive bees were treated. Row 1 of Panel (B) in which only hive bees are treated and Row 2 in Panel (B) in which the most stressed forager bees are treated demonstrate the importance of foragers to the colony: a slight decrease in the amount of necessary recovery time results from the effect of social regulation from foragers aiding in the colony. This agrees with the Khoury model because the increase in the death rate of foragers was observed to result in a death spiral [14]. The category for which treatment yields the best effect is the least stressed foragers (Rows 3-4). However, a comparison of the respective outcomes of treating the most stressed forager bees (Row 2) and the most stressed hive bees (Column 2) reveals little difference, indicating that prioritizing treatment of the least stressed bees of both categories is preferred over treating the most stressed bees of either category. Finally, the best outcome comes from treating both categories of bees with extreme treatment (Row 4, Column 4). However, practically the same result can be achieved by simply treating all bees (Row 3); the difference is a matter of a fraction of days that does not ultimately have a material effect on a beekeeping operation.

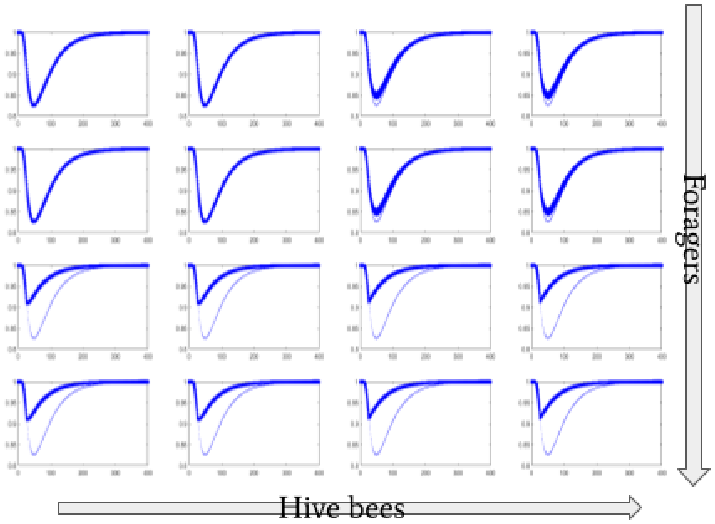
The amount of time that the treatments are given for are: 0 days (equivalent to never treating); 1 day; 2 days; 5 days; 1 week (7 days); 2 weeks (14 days); and a full year (365 days). The effectiveness of treatments increases with the amount of time in which the treatment is applied. The most significant difference is between no treatment time and any amount of treatment time. However, as the amount of treatment time increases, the added effectiveness is not materially significant. Typically, a week of treatment yields similar results to that of a year of treatment. The amounts were chosen for ease of reference.

In Figure 4, we show our model outcomes for Scenario 2 in Table 3. This simulation is for a pollination job that is for half a month, where the colony is able to commence treatment 1 day after the pollination job ends and has a high stressor importation rate, and the hive bees have a natural ability to clean the stressor. Similar to Scenario 1, we note that the best outcomes are found when foragers that are of the least stressed category are treated. We also note that treatment of H bees only (Row 1, Columns 2-4) does not change the outcome much from “no treatment” (Row 1, Column 1). In addition, there is a difference between treating only forager bees (Column 1) and treating both

(A)



(B)



(C)

Min size (%)	82.61	82.61	84.98	84.98	82.62	82.62	84.98	84.98	91.04	91.04	91.37	91.36	91.04	91.04	91.37	91.36
Min time (day)	49	49	50	50	49	49	50	50	30	30	27	27	30	30	27	27
Time 95%	112	112	104	104	112	112	104	104	65	65	56	56	65	65	56	56
Time 99%	196	195	188	188	196	196	188	188	148	148	139	140	148	148	139	139

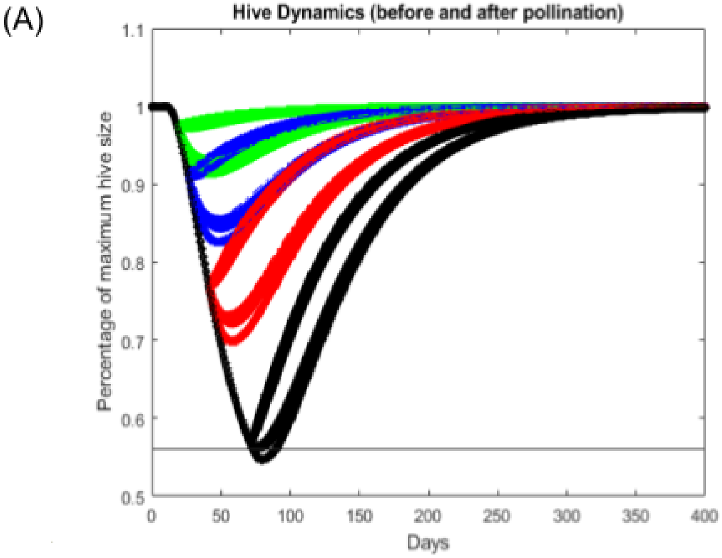
Figure 4: In Figure 4, we consider Scenario 2 from Table 3. Here, we allow for pollination jobs of 1 week, half a month, a month, and two months in length, corresponding to the green, blue, red and black colours in Panel (A). We then consider sixteen scenarios for treatment (see Table 3), where treatment is commenced on day 16 after the end of the pollination job. In Panels (B) and (C), we consider the half–month-long pollination job scenario only (corresponding to the blue curves in Panel (A)). In Panel (B), we see that, intuitively, "no treatment" is the worst scenario (the thin blue line that reaches the minimum population value). However, we see that treatment will benefit the colony, reducing the recovery time of the colony back to maximum size. The largest reductions in recovery time correspond to treatments that preferentially treat forager bees F. The worst (red text) and best (green text) case scenarios for treatment are highlighted in Table (C). Overall, the best outcome corresponds to a minimum size of approximately 91%, which is reached at day 27, 1 day after the initiation of and 2 days after the end of the pollination job. We also observe that the colony reaches 95% and 99% of maximal colony size 30 and 113 days after the end of the pollination job (corresponding to days 56 and 139 of the simulation). In some cases, the minimum size of a treated colony is more than 95%. There is very little diversity in terms of outcomes related to treatment duration. However, there is a difference between no treatment and treatment of all castes of bees. In addition, there is a difference between treating only forager bees and treating both hive and forager bees. Treatment has the potential to reduce recovery time by around 2 months.

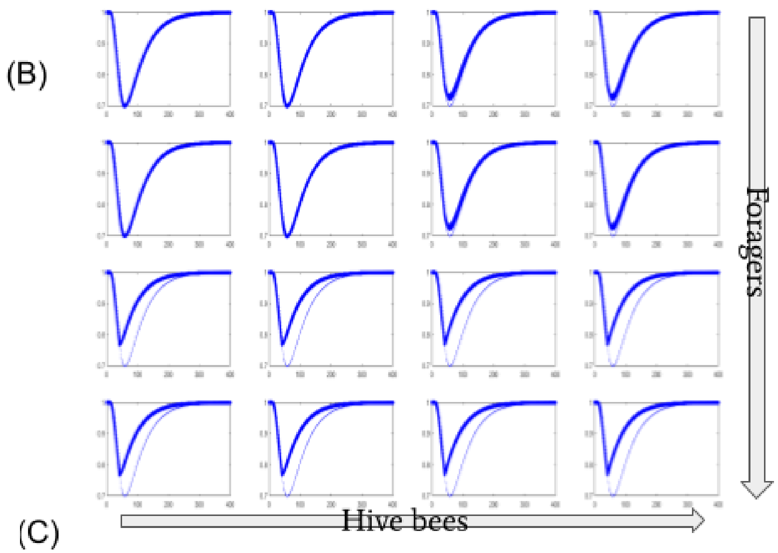
hive and forager bees (Columns 2-4). Treatment has the potential to reduce recovery time by around 2 months (Panel (C)).

In Figure 5, we present simulations for Scenario 3 (Table 3), a pollination job that lasts a month, where the colony is able to start treatment 1 day later, has a high stressor importation rate, and the hive bees have a natural ability to clean the stressor. Again, similar to Scenario 1, we note that the best outcomes are found when foragers that are of the least stressed category are treated. We also note that treatment of H bees only (Row 1, Columns 2-4) does not change the outcome much from "no treatment" (Row 1, Column 1). In addition, there is a difference between treating only forager bees (Column 1) and treating both hive and forager bees (Columns 2-4). Treatment has the potential to reduce recovery time by around 2 months (Panel (C)). Figure 5 also shows that there is little diversity in outcomes for different lengths of treatments. A longer treatment length most benefits the colony, but a treatment length of a week (7 days) can achieve similar outcomes in most of the panels found in Section (B).

A month is the most commonly reported length of a pollination job [4]. Therefore, our simulations in Figure 5 could indicate that for a pollination job in which the stressor is dangerous, but can be properly processed by the colony, the only factor that is significant is if treatment is applied at all. The best case scenario of treatment reduces recovery time by around a month (Row 3-4, Columns 3-4).

In Figure 6, we present simulations for Scenario 4 (Table 3), a pollination job that is for two months, where the colony is able to start treatment 1 day later, has a high stressor importation rate, and the hive bees have a natural ability to clean the stressor. There is little diversity in outcomes for treatment lengths, and the bee population eventually goes below the approximate 60% threshold needed to maintain the colony for winter before recovering. The best

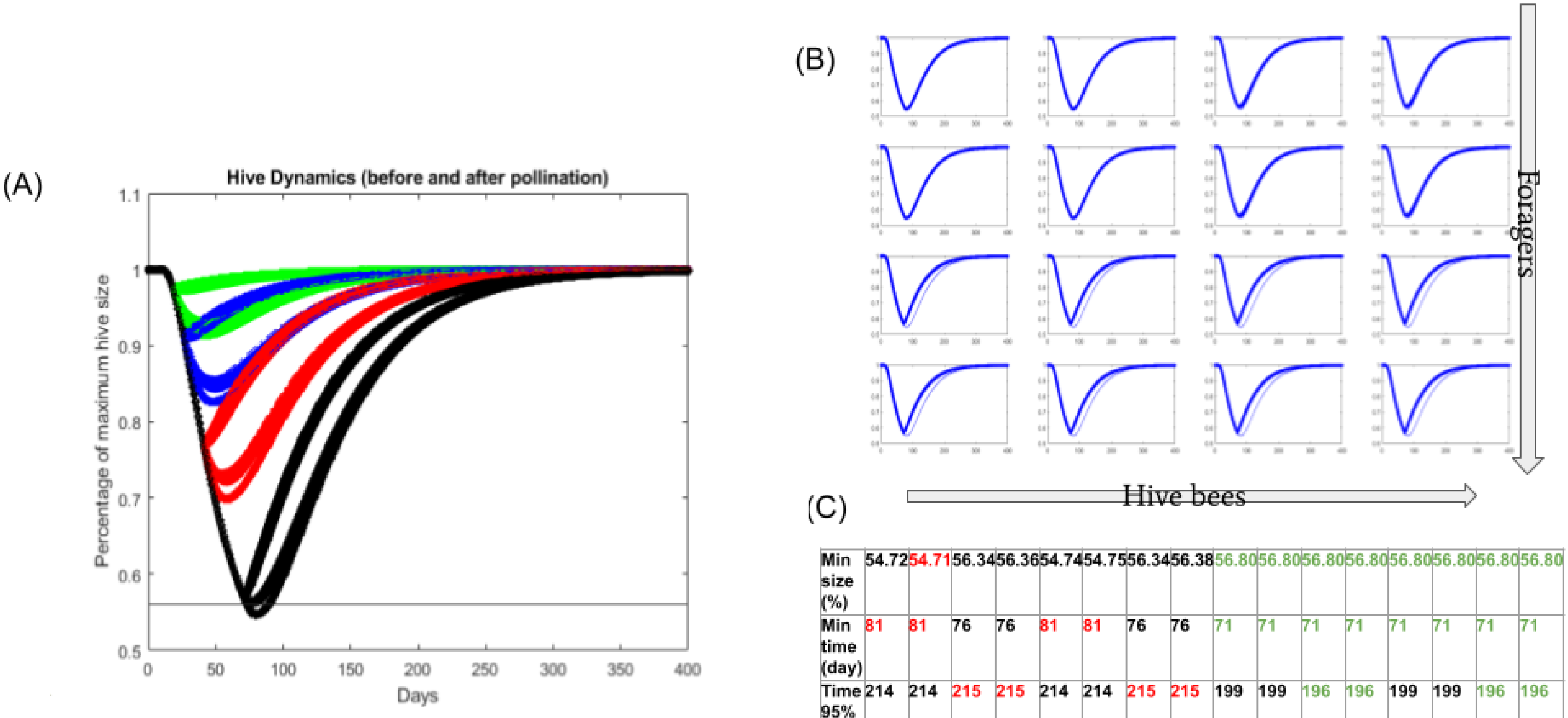




(C)

Min size (%)	69.87	69.85	72.83	72.82	69.91	69.87	72.86	72.88	76.84	76.85	76.96	76.96	76.86	76.85	76.96	76.96
Min time (day)	59	59	57	57	59	59	57	57	42	42	41	41	42	42	41	41
Time 95%	158	158	154	154	158	158	154	154	132	132	126	126	131	131	126	126
Time 99%	242	242	237	238	242	242	237	237	215	215	210	210	215	215	210	210

Figure 5: In Figure 5, we consider Scenario 3 from Table 3. Here, we allow for pollination jobs of 1 week, half a month, a month, and two months in length, corresponding to the green, blue, red and black colours in Panel (A). We then consider sixteen scenarios for treatment (see Table 3), where treatment is commenced on day 41 after the end of the pollination job. In Panels (B) and (C), we consider the month-long pollination job scenario only (corresponding to the red curves in Panel (A)). In Panel (B), we see that, intuitively, “no treatment” is the worst scenario (the thin blue line that reaches the minimum population value). However, we see that treatment will benefit the colony, reducing the recovery time of the colony back to maximum size. The largest reductions in recovery time correspond to treatments that preferentially treat forager bees F. The worst (red text) and best (green text) case scenarios for treatment are highlighted in Table (C). Overall, the best outcome corresponds to a minimum size of approximately 77%, which is reached at day 41, 1 day after the initiation of and 1 day after the end of the pollination job. We also observe that the colony reaches 95% and 99% of maximal colony size 86 and 170 days after the end of the pollination job (corresponding to days 126 and 210 of the simulation). In some cases, the minimum size of a treated colony is more than 95 percent. There is little diversity in outcomes for lengths of treatments. A month is the most commonly reported length of a pollination job. This could indicate that for a pollination job in which the stressor is dangerous, but can be properly processed by the colony, the only factor that is significant is if treatment is applied at all. The best case scenario of treatment reduces recovery time by around a month.



(C)

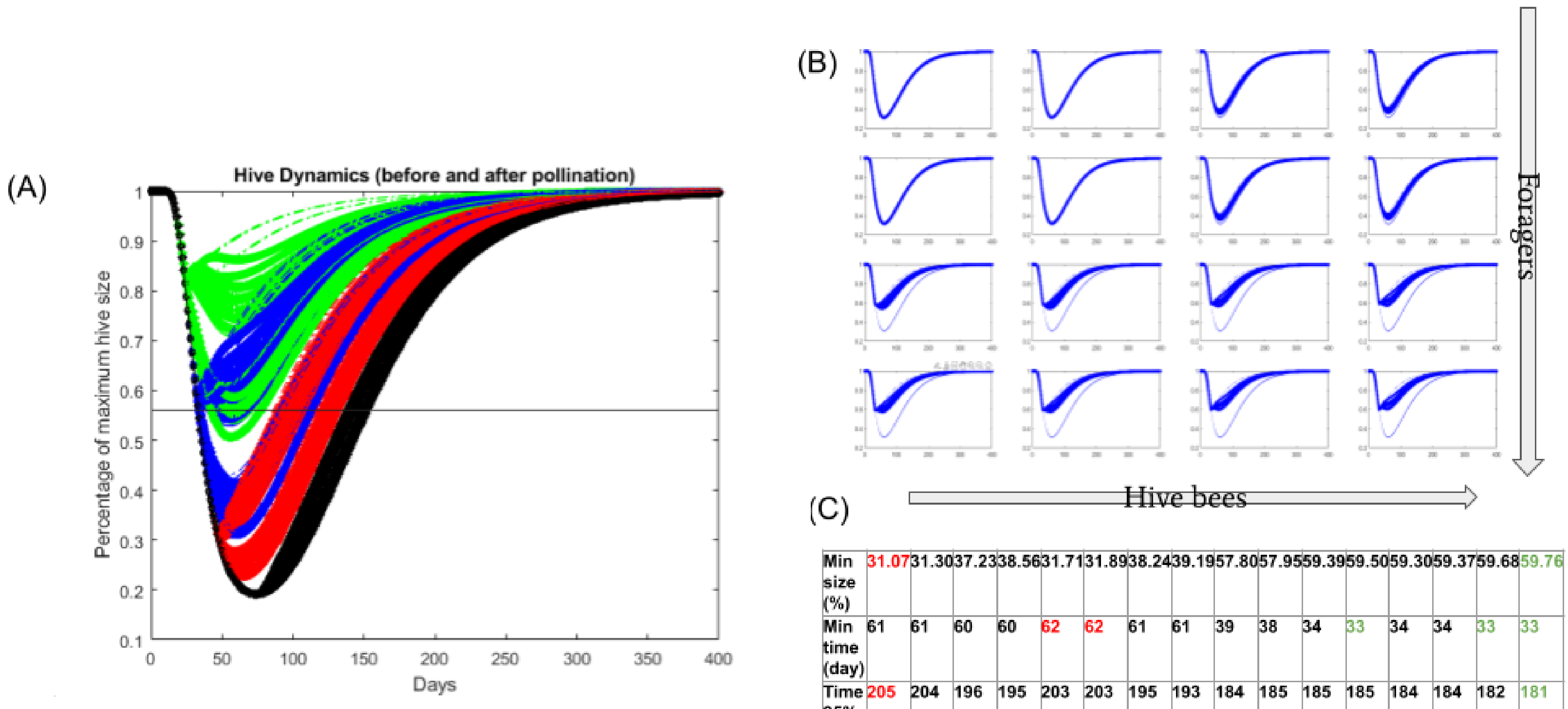
Min size (%)	54.72	54.71	56.34	56.36	54.74	54.75	56.34	56.38	56.80	56.80	56.80	56.80	56.80	56.80	56.80	56.80
Min time (day)	81	81	76	76	81	81	76	76	71	71	71	71	71	71	71	71
Time 95%	214	214	215	215	214	214	215	215	199	199	196	196	199	199	196	196
Time 99%	298	298	298	298	298	298	298	298	283	283	280	280	283	283	279	279

Figure 6: In Figure 6, we consider Scenario 4 from Table 3. Here, we allow for pollination jobs of 1 week, half a month, a month, and two months in length, corresponding to the green, blue, red and black colours in Panel (A). We then consider sixteen scenarios for treatment (see Table 3), where treatment is commenced on day 71 after the end of the pollination job. In Panels (B) and (C), we consider the week-long pollination job scenario only (corresponding to the green curves in Panel (A)). In Panel (B), we see that, intuitively, "no treatment" is the worst scenario (the thin blue line that reaches the minimum population value). However, we see that treatment will benefit the colony, reducing the recovery time of the colony back to maximum size. The largest reductions in recovery time correspond to treatments that preferentially treat forager bees F. The worst (red text) and best (green text) case scenarios for treatment are highlighted in Table (C). Overall, the best outcome corresponds to a minimum size of approximately 57%, which is reached at day 71, on the day of the initiation of and 1 day after the end of the pollination job. We also observe that the colony reaches 95% and 99% of maximal colony size 126 and 209 days after the end of the pollination job (corresponding to days 196 and 279 of the simulation). In some cases, the minimum size of a treated colony is more than 95 percent. There is little diversity in outcomes for treatment lengths, and the bee population eventually goes below the approximate 60% threshold needed to maintain the colony for winter before recovering. The best case treatment reduces recovery time by around 3 weeks. Comparing this to Figure 5, we start to observe that there is a cost to waiting for the treatment to be applied.

case treatment reduces recovery time by around 3 weeks. Comparing this to Figure 5, we start to observe that there is a cost to waiting for the treatment to be applied.

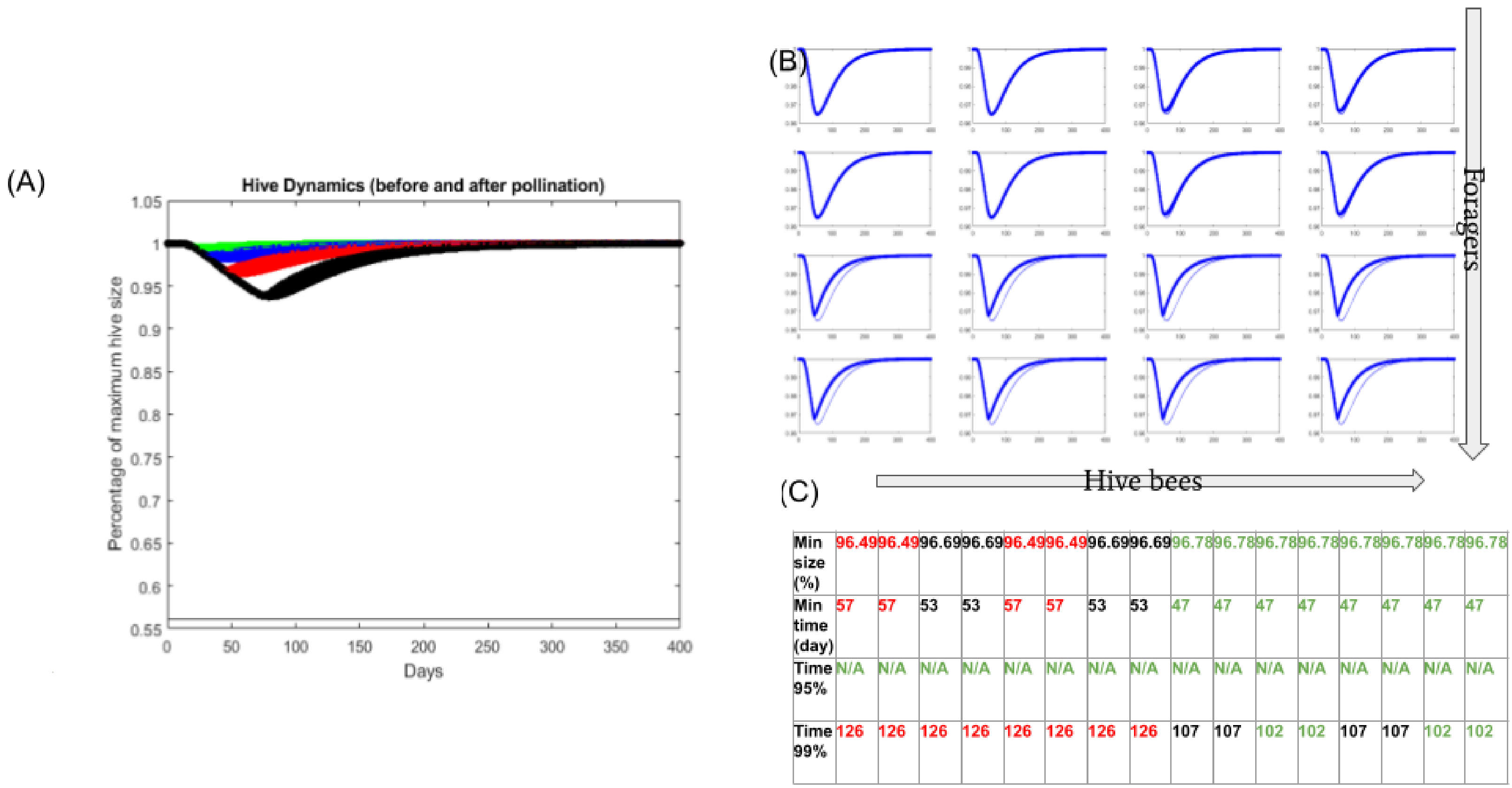
In Figure 7, we present simulations for Scenario 5 (Table 3), a pollination job that is for half a month, where the colony is able to start treatment one week later, has a high stressor importation rate, and the bees have no natural ability to clean the stressor. The best case recovery time for a week of treatment reduces recovery time by around 3 weeks. This shows that delaying the application of a treatment also has a negative effect on the utility of treatment. Even though the job ended earlier, there was a clear difference between Figure 5 and Figure 7 in terms of effectiveness of a pollination job. Figure 7 shows a clear disadvantage, suggesting that treatment should not be delayed.

In Figure 8, we present simulations for Scenario 6 (Table 3), a pollination job that lasts a month, where the colony is able to start treatment one week later, has a low stressor importation rate, and the hive bees have a natural ability to clean the stressor. There is not much diversity in outcomes for treatment length or treatment types, as the situation is still ideal with a low stressor importation rate and natural cleaning. There is a diversity of outcomes for treatment durations for all castes of bees. In both simulations, there is a difference between treating only the least stressed bees and treating all categories of bees. In the treatments of only foragers, this difference is significant because there are outcomes in simulations for which the least stressed bees are treated that result in the colony going below the 60 percent threshold needed to survive winter. The only treatments which guarantee that the colony never goes below the 60 percent threshold are ones in which both castes of bees are treated and at minimum all forager bees are treated. The best case recovery time for a week of treatment reduces recovery time by around 3 weeks.



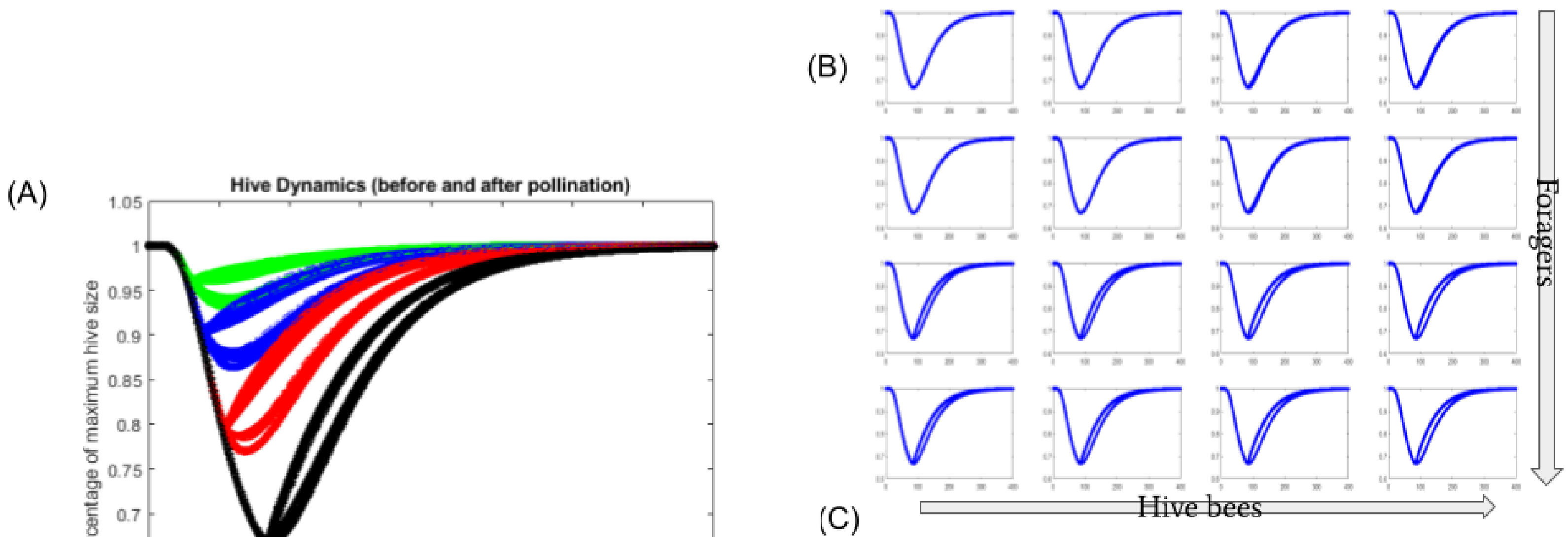
Min size (%)	31.07	31.30	37.23	38.56	31.71	31.89	38.24	39.19	57.80	57.95	59.39	59.50	59.30	59.37	59.68	59.76
Min time (day)	61	61	60	60	62	62	61	61	39	38	34	33	34	34	33	33
Time 95%	205	204	196	195	203	203	195	193	184	185	185	185	184	184	182	181
Time 99%	288	288	280	278	287	287	278	277	267	268	268	268	268	268	265	264

Figure 7: In Figure 7, we consider Scenario 5 from Table 3. Here, we allow for pollination jobs of 1 week, half a month, a month, and two months in length, corresponding to the green, blue, red and black colours in Panel (A). We then consider sixteen scenarios for treatment (see Table 3), where treatment is commenced 1 day after the end of the pollination job. In Panels (B) and (C), we consider the half-a-month-long pollination job scenario only (corresponding to the blue curves in Panel (A)). In Panel (B), we see that, intuitively, "no treatment" is the worst scenario (the thin blue line that reaches the minimum population value). However, we see that treatment will benefit the colony, reducing the recovery time of the colony back to maximum size. The largest reductions in recovery time correspond to treatments that preferentially treat forager bees F. The worst (red text) and best (green text) case scenarios for treatment are highlighted in Table (C). Overall, the best outcome corresponds to a minimum size of approximately 60%, which is reached at day 33, 11 days after the initiation of and 18 days after the end of the pollination job. We also observe that the colony reaches 95% and 99% of maximal colony size 166 and 249 days after the end of the pollination job (corresponding to days 181 and 264 of the simulation). In some cases, the minimum size of a treated colony is more than 95%. The best case recovery time for a week of treatment reduces recovery time by around 3 weeks. This shows that delaying the application of a treatment also has a negative effect on the utility of treatment. Even though the job ended earlier, there was a clear difference between Figure 5 and Figure 7 in terms of effectiveness of a pollination job. Figure 7 shows a clear disadvantage, suggesting that treatment should not be delayed.



Min size (%)	96.49	96.49	96.69	96.69	96.49	96.49	96.69	96.69	96.78	96.78	96.78	96.78	96.78	96.78	96.78	96.78
Min time (day)	57	57	53	53	57	57	53	53	47	47	47	47	47	47	47	47
Time 95%	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Time 99%	126	126	126	126	126	126	126	126	107	107	102	102	107	107	102	102

Figure 8: In Figure 8, we consider Scenario 6 from Table 3. Here, we allow for pollination jobs of 1 week, half a month, a month, and two months in length, corresponding to the green, blue, red and black colours in Panel (A). We then consider sixteen scenarios for treatment (see Table 3), where treatment is commenced 7 days after the end of the pollination job. In Panels (B) and (C), we consider the month-long pollination job scenario only (corresponding to the red curves in Panel (A)). In Panel (B), we see that, intuitively, "no treatment" is the worst scenario (the thin blue line that reaches the minimum population value). However, we see that treatment will benefit the colony, reducing the recovery time of the colony back to maximum size. The largest reductions in recovery time correspond to treatments that preferentially treat forager bees F. The worst (red text) and best (green text) case scenarios for treatment are highlighted in Table (C). Overall, the best outcome corresponds to a minimum size of approximately 97%, which is reached at day 47, 9 days after the initiation of and 2 days after the end of the pollination job. We also observe that the colony never goes below 95% of maximum colony size and reaches 99% of maximal colony size 62 days after the end of the pollination job (corresponding to days 40 and 102 of the simulation). In some cases, the minimum size of a treated colony is more than 95%. There is not much diversity in outcomes for treatment length or treatment types, as the situation is still ideal with a low stressor importation rate and natural cleaning. There is a diversity of outcomes for treatment durations for all castes of bees. In both simulations, there is a difference between treating only the least stressed bees and treating all categories of bees. In the treatments of only foragers, this difference is significant because there are outcomes in simulations for which the least stressed bees are treated that result in the colony going below the 60 percent threshold needed to survive winter. The only treatments which guarantee that the colony never goes below the 60 percent threshold are ones in which both castes of bees are treated and at minimum all forager bees are treated.



(C)

Min size (%)	66.86	66.86	66.95	66.95	66.86	66.86	66.95	66.95	66.95	66.95	66.95	66.95	66.95	66.95	66.95	66.95
Min time (day)	87	87	84	84	87	87	84	84	84	84	84	84	84	84	84	84
Time 95%	199	199	199	199	199	199	199	199	184	184	182	182	184	184	182	182
Time 99%	282	282	282	282	282	282	282	282	268	268	265	265	268	268	265	265

Figure 9: In Figure 9, we consider Scenario 7 from Table 3. Here, we allow for pollination jobs of 1 week, half a month, a month, and two months in length, corresponding to the green, blue, red and black colours in Panel (A). We then consider sixteen scenarios for treatment (see Table 3), where treatment is commenced 14 days after the end of the pollination job. In Panels (B) and (C), we consider the two-month-long pollination job scenario only (corresponding to the black curves in Panel (A)). In Panel (B), we see that, intuitively, “no treatment” is the worst scenario (the thin blue line that reaches the minimum population value). However, we see that treatment will benefit the colony, reducing the recovery time of the colony back to maximum size. The largest reductions in recovery time correspond to treatments that preferentially treat forager bees F. The worst (red text) and best (green text) case scenarios for treatment are highlighted in Table (C). Overall, the best outcome corresponds to a minimum size of approximately 67%, which is reached at day 84, on the day of treatment initiation and 14 days after the end of the pollination job. We also observe that the colony reaches 95% and 99% of maximal colony size 112 and 195 days after the end of the pollination job (corresponding to days 182 and 265 of the simulation). In some cases, the minimum size of a treated colony is more than 95 percent. There is only a slight diversity in treatment outcomes and not much difference in treatment outcomes across all castes. The best case recovery time for a week of treatment reduces recovery time by around 2 weeks. Interestingly enough, this is only around a week's difference from Figure 6. This means that an importation rate that is 10 times the amount needed to stress out a bee will not translate linearly to a decrease in recovery time.

In Figure 9, we present simulations for Scenario 7 (Table 3), a pollination job lasts two months, where the colony is able to start treatment two weeks later, has a low stressor importation rate, and the hive bees have a natural ability to clean the stressor. There is only a slight diversity in treatment outcomes and not much difference in treatment outcomes across all castes. The best case recovery time for a week of treatment reduces recovery time by around 2 weeks. Interestingly enough, this is only around a week difference from Figure 6. This means that an importation rate that is 10 times the amount needed to stress out a bee will not translate linearly to a decrease in recovery time.

In Figure 10, we present simulations for Scenario 8 (Table 3), a pollination job that lasts a week, where the colony is able to start treatment 1 day later, has an extremely high stressor importation rate, and the bees have no natural ability to clean the stressor. The colony goes below 0.001 per cent of its maximum colony size, which should be treated as a total collapse of the colony. "No treatment" is effective from a practical standpoint. The colony is beyond salvaging in this scenario. The fact that this scenario was able to occur not only means that the beekeeper's colony was destroyed, but also that they wasted money on testing. Although there were scenarios in which the Jacobian matrix did not have a positive eigenvalue at the origin, this was not one such scenario. This means that although the extinction condition of zero bees was unstable, the stable population was still low enough to have effectively a dead colony. We note that stochastic effects might prevent a queen from causing the colony to rebound, which would not be included in a system of ordinary differential equations.

The lowest amount of time needed to recover to 99% of maximum colony size from each scenario was in Scenario 1 (Table 3) when every caste received the necessary treatment, and where the amount of time needed to recover after the pollination job was 56 days in total.

The largest recovery time for the colony was in Scenario 8 (Table 3) where recovery was not achieved within the 400 days allowed in the simulation. However, in terms of measurable recovery, Scenario 5 (Table 3) was the highest at 274 days needed for a colony to recover when no treatment was applied. In addition to the best case scenario where treatment can also be applied, Scenario 5 also has the largest time needed to recover at 251 days.

4 Discussion

4.1 Conclusions

We have compared various scenarios that a honeybee colony may face when undergoing a pollination job and then recovering from that pollination job. We have compared the recovery times in scenarios with varying treatment outcomes and stressor levels, as well as the ability of the honeybees to naturally dispose of the stressor. The most important comparison we did was between the times when the treatment was applied and the duration of the treatment.

In scenarios in which treatment can be applied to only the bees of the lowest stress level, treatment is comparable to treating all bees. In the scenarios in which treatment can be applied to only bees of the highest stress level, treatment is comparable to treating no bees. It is not known whether a stressor will be able to be treated in the most optimal way. However, across all treatment types, results of treating for a 14-day period are comparable to treating for a 365-day period. But importantly, treatment becomes less and less effective as it is delayed. Moreover, the amount of bees available for treatment and recovery similarly decreases over time as a smaller population is available for treatment. In addition to this, it is possible in some scenarios to allow exposure equivalent to a few pollination jobs while still maintaining reasonable colony numbers. In scenarios with extreme stressor importation and stressor effect (i.e., extreme pesticide use), it is not feasible to treat at all due to the collapse of a colony. Whether the bees were exposed to a higher or lower amount of the stressor was a large factor due to a tenfold increase in stressor importation resulting in different outcomes. However, it seems that the ability of the bees to enzymatically remove (or “clean”) the stressor is highly significant due to it impacting how effective treatment is, in that it significantly increases the effectiveness of treatment. It was found that of the parameters being altered within the system, the most crucial was the time at which treatment started after a pollination job. The other factors performed as expected when manipulated, with higher pesticide importation rates, mortality rates, and lower pesticide disposal resulting in a large decline for the colony.

The best case scenario for colony recovery that we tested for was Scenario 6 due to the fact that there were scenarios that never even went below 95% maximum colony size. Every other scenario went below 95%. This means that in the presence of bees being able to naturally clean the stressor, and a low enough stressor importation rate, there would be no practical value in treatment.

For scenarios which depict a colony size that goes below 95%, the best case scenario is Scenario 2, which has the best outcome between treatment and non-treatment at around 2 months saved in recovery time.

The worse case scenario for colony survival is Scenario 8 in which the stressor importation rate was extremely high, to the point of practical extinction for the colony. However, in terms of a case that was not extreme, the worst outcome was Scenario 5. In Scenario 5, there was no natural way to dispose of the stressor and the importation rate was ten times the amount of Scenario 6. In contrast with Scenario 6, where the colony population never dips below 95%, Scenario 5 has multiple situations in which the population goes below the minimum amount of bees needed to survive the winter. Scenario 5 also has much more variance in the outcomes of the lengths of treatment. In most of the scenarios shown, there is not much variance between outcomes, meaning that a treatment should only be applied for a few days until natural recovery. However, it seems that a high amount of stressor importation and the inability to naturally dispose of the stressor may necessitate a longer period of applying treatment. That said, even with Scenario 5's conditions, there is very little difference between 2 weeks of treatment and a year of treatment.

Our model has certain limitations that do not affect its results. Limitations of our model include that it involves only one simplified stressor and only considers two castes of honeybees. The brood, reproductive castes or differentiated castes are not examined because the mechanics of stressors affecting them are not included in the study. The brood and reproductive castes would necessitate the inclusion of delay terms from the developing brood, stochastic effects from the singular queen, and drifting behaviour from the mobile drones to accurately capture all behaviour.

Our model demonstrates that "early" should be the watchword for testing. It also shows that trying to save the bees at higher levels of stress is not warranted due to the fact that there is negligible utility in treating at those levels.

4.2 Future research

Our model uses a generic stressor which is harmful, but does not spread. Even situations in which it can decay and leave the colony can result in the utter deterioration of the colony's health. Diseases and parasites such as *Nosema* can still remain in the colony for long periods of time and spread between colonies [19]. Multi-colony analysis for a generic stressor would be useful for determining the average impact that testing on an operation based on proximity to a stressor would have. However, diseases would necessitate taking into account the added factor of the disease likely afflicting the entirety of the beekeeping operation. One other potential interest is the possible existence of a vaccine for diseases in bee colonies [21].

Another possible simulation study would be comparing the testing of two different stressors to determine which stressors are more sensitive to earlier testing and which combinations of stressor testing would be most effective. It has been found that pesticides can have a multiplicative effect on bee mortality. A beekeeping operation with multiple colonies could potentially use this model to make decisions on whether to treat or merge colonies based on pesticide exposure levels. In addition, multiple simulations of the model could be used to perform a random forest model to determine a best practice for the bee colonies. Such a simulation would also have to take into account the stochastic effects caused by the probability of a queen dying and necessitating colony mergers.

Further, our model is not entirely coupled with the agricultural sector, as different crops have different pollination needs [2]. Our model could be adapted to study crops on a case by case basis in order to see the secondary impact of bee health on the output of crops. A major component in determining effectiveness of a given crop would be the amount of bee visits to the flowers, the sex of the flowers, and the amount of pesticide commonly used for a given crop.

Overall, our model gives a framework for the effect of testing and treating a stressed colony. There were situations in which the colonies were either too far gone to benefit from treatment, such as Scenario 8, or too well off to need treatment, such as Scenario 6. Treatment is only needed until the natural recovery of the colony activates. As well, two weeks of treatment can be close to optimal. This is also easy to remember for beekeepers. The main limit to the effectiveness of treatment seems to be that any treatment can only save as many bees as those that have survived up until the point of discovery of the stressor. Nevertheless, the overarching constant remains that the earlier the treatment was applied, the more effective the outcome.

References

[1] Statistical Overview of the Canadian Honey and Bee Industry and the Economic Contribution of Honey Bee Pollination, 2016 - agriculture.canada.ca — agriculture.canada.ca. https://agriculture.canada.ca/en/sector/horticulture/reports/statistical-overview-canadian-honey-and-bee-industry-and-economic-contribution-honey-bee-pollinationa5.2. [Accessed 28-Jun-2023].

[2] The Daily Production and value of honey, 2022 — www150.statcan.gc.ca. https://www150.statcan.gc.ca/n1/daily-quotidien/221214/dq221214f-eng.htm. [Accessed 28-Jun-2023].

[3] Marcelo Aizen and Lawrence Harder. The Global Stock of Domesticated Honey Bees Is Growing Slower Than Agricultural Demand for Pollination. *Current biology : CB*, 19:915–8, 06 2009.

[4] BeeCSI. unpublished BeeCSI survey, howpublished = , 2022. [Accessed 28-Jun-2023].

[5] Brown, Mark J.F. and Paxton, Robert J. The conservation of bees: a global perspective. *Apidologie*, 40(3):410–416, 2009.

[6] Jae. C Choe. *Encyclopedia of Animal Behavior.* Academic Press, Cambridge, Massachusetts, 2019.

[7] John Connor Lawrence. *Honey Bee Biology and Beekeeping, Revised Edition*. Wicwas Press, Kalamazoo, Michigan, 2013.

[8] Athel Cornish-Bowden. Current iubmb recommendations on enzyme nomenclature and kinetics. *Perspectives in Science*, 1:74–87, 05 2014.

[9] Lucas Garibaldi, Luísa Carvalheiro, Bernard Vaissière, Barbara Gemmill-Herren, Juliana Hipólito, Breno Freitas, Hien T. Ngo, Nadine Azzu, Agustin Saez, Jens Åström, Jiandong An, Betina Blochtein, Damayanti Buchori, Fermin Chamorro, Fabiana Silva, Kedar Devkota, Márcia Ribeiro, Leandro Freitas, Maria Cristina Gaglianone, and Hong Zhang. Mutually beneficial pollinator diversity and crop yield outcomes in small and large farms. *Science*, 351:388–391, 01 2016.

[10] Gerhard Heldmaier. Temperature control in honey bee colonies. *BioScience*, 37:395–399, 06 1987.

[11] Michelle Hladik, Anson Main, and Dave Goulson. Environmental Risks and Challenges Associated with Neonicotinoid Insecticides. *Environmental Science Technology*, 52, 02 2018.

[12] James Keener and James Sneyd. *Mathematical physiology. I: Cellular physiology. 2nd ed*, volume 8/1. 01 2008.

[13] David S. Khoury, Andrew B. Barron, and Mary R. Myerscough. Modelling Food and Population Dynamics in Honey Bee Colonies. *PLOS ONE*, 8(5):1–7, 05 2013.

[14] David S Khoury, Mary R Myerscough, and Andrew B Barron. A quantitative model of honey bee colony population dynamics. *PloS one*, 6(4):e18491, 2011.

[15] Pierre Magal, Glenn F. Webb, and Yixiang Wu. An environmental model of honey bee colony collapse due to pesticide contamination. *Bulletin of Mathematical Biology*, 81:4908 – 4931, 2019.

[16] Alex Petric, Ernesto Guzman, and Hermann Eberl. A mathematical model for the interplay of nosema infection and forager losses in honey bee colonies. *Journal of Biological Dynamics*, 11:1–31, 10 2016.

[17] Vardayani Ratti, Peter Kevan, and Hermann Eberl. A mathematical model of forager loss in honeybee colonies infested with varroa destructor and the acute bee paralysis virus. *Bulletin of Mathematical Biology*, 79:1218–1253, 04 2017.

[18] Gene Robinson. Regulation Of Division Of Labor In Insect Societies. *Annual review of entomology*, 37:637–65, 02 1992.

[19] Avitabile Sammataro. *The Beekeeper's Handbook Fourth Edition*. Cornell University Press, Sage House, 512 East State Street, Ithaca, New York 14850, 2011.

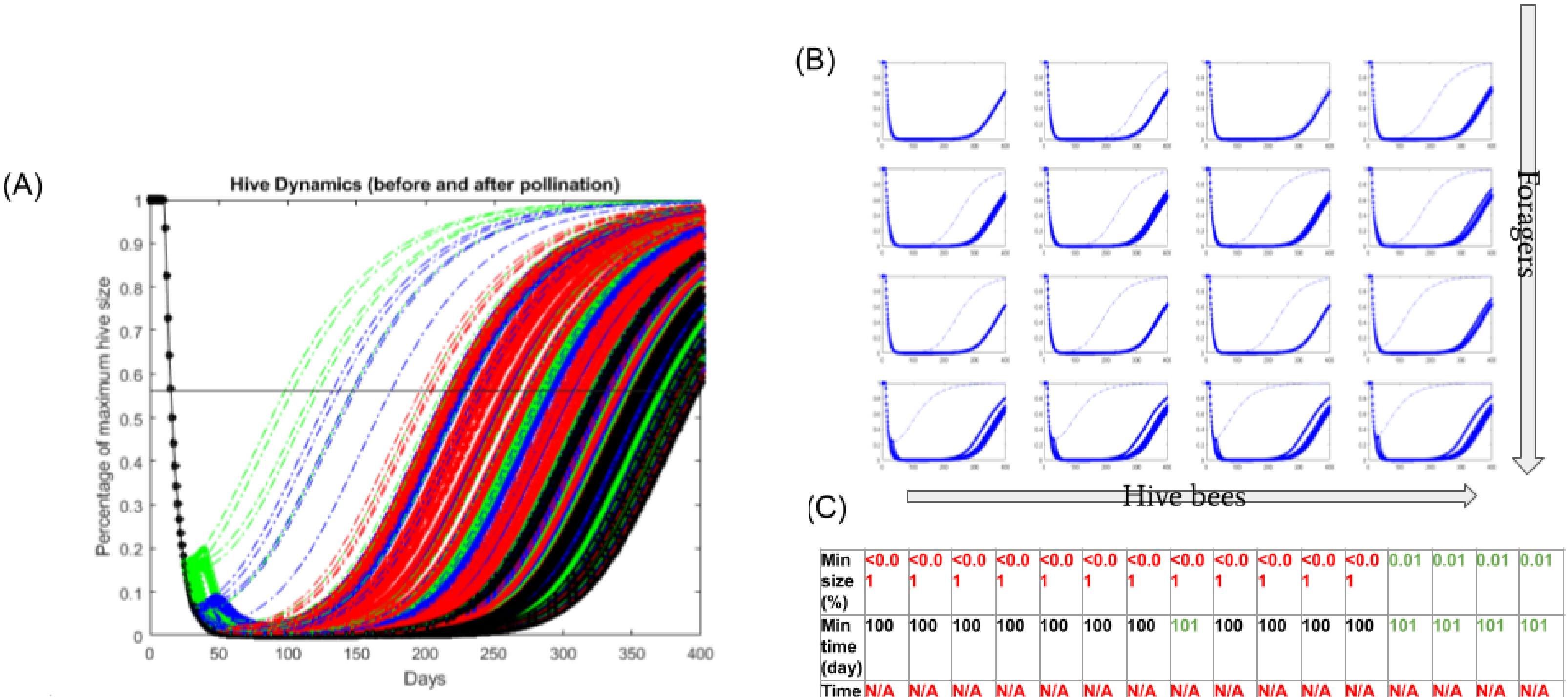
[20] Ricard Sole, Eric Bonabeau, Jordi Delgado, Pau Fernandez, and Jesús Marín. Pattern formation and optimization in army ant raids. *Artificial life*, 6:219–26, 02 2000.

[21] Remy Tumin. U.s.d.a. approves first vaccine for honeybees. *New York Times*.

[22] Dennis vanEngelsdorp, Jay D. Evans, Claude Saegerman, Chris Mullin, Eric Haubruge, Bach Kim Nguyen, Maryann Frazier, Jim Frazier, Diana Cox-Foster, Yanping Chen, Robyn Underwood, David R. Tarpy, and Jeffery S. Pettis. Colony Collapse Disorder: A Descriptive Study. *PLOS ONE*, 4(8):1–17, 08 2009.

[23] Andrea Wade, Chia-Hua Lin, Colin Kurkul, Erzsébet Regan, and Reed Johnson. Combined Toxicity of Insecticides and Fungicides Applied to California Almond Orchards to Honey Bee Larvae and Adults. *Insects*, 10:20, 01 2019.

[24] Gordon Wardell, Peter Bernhardt, Ron Bitner, Alberto Burquez, Stephen Buchmann, Jim Cane, Paul Cox, Virginia Dalton, Peter Feinsinger, Mrill Ingram, David Inouye, C. Jones, Kathryn Kennedy, Peter Kevan, Harold Koopowitz, Rodrigo Medellín, Medellín Morales, Gary Nabhan, Bruce Pavlik, and Steve Walker. The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology*, 12:8–17, 02 1998.



(C)

Min size (%)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01	0.01	0.01
Min time (day)	100	100	100	100	100	100	100	101	100	100	100	100	101	101	101	101
Time 95%	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Time 99%	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Figure 10: In Figure 10, we consider Scenario 8 from Table 3. Here, we allow for pollination jobs of 1 week, half a month, a month, and two months in length, corresponding to the green, blue, red and black colours in Panel (A). We then consider sixteen scenarios for treatment (see Table 3), where treatment is commenced on day 1 after the end of the pollination job. In Panels (B) and (C), we consider the week-long pollination job scenario only (corresponding to the green curves in Panel (A)). In Panel (B), we see that treatment has effects on recovery, but that the minimum colony size achieved would be less than 3 bees for all scenarios. Practically speaking, this would indicate a collapsed colony and therefore rule out the possibility of a recovery.