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Research article

Mathematical modelling of population and food storage dynamics in a honey bee colony infected with *Nosema ceranae*

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ABSTRACT

Unusually high wintering losses of *Apis mellifera* in recent years has raised concerns regarding the well-being and productivity of honey bees across the globe. While these losses are likely multi-factorial, a proposed contributor are diseases, including those caused by parasites. We formulate and present a mathematical model for a colony of *Apis mellifera* honey bees infected with the microsporidian parasite *Nosema ceranae*. The model is numerically analyzed to determine the effects of *N. ceranae* infection on population and food storage dynamics and their subsequent implications towards colony survival and annual honey yield. Depending on the strength of disease, it is possible for either parasite fadeout, co-existence between bees and *N. ceranae*, or colony failure to occur. In all cases, the yield of honey collected by the beekeeper is reduced. We further extend the model to include various treatment schemes with the, now discontinued, antimicrobial fumagillin. Treatment with fumagillin can reduce the risk of colony failure and will increase honey yield compared to when no treatment is applied.

1. Introduction

Overview. The Western honey bee, *Apis mellifera*, has been threatened in recent years due to a number of proposed stressors including poor climate and weather, pesticides and disease [1]. As a result, unusually high wintering losses have been observed in many regions of North America and Europe. For example, between 2007 and 2017, Canadian beekeepers experienced an average 25% colony loss over winter with losses ranging between 15.3% and 35% [2]. It was in 2008 that the parasite *Nosema ceranae* was suggested as the cause of such drastic colony losses [3]. Since then, the role of *N. ceranae* in colony failure has been studied extensively but results have been inconsistent. While there is some evidence to support the hypothesis that *N. ceranae* is a key component in failing colonies [3, 4, 5], other studies have presented contradictory evidence [6, 7, 8, 9].

Whether a colony survives or fails, however, is not the only outcome of interest for beekeepers. The overall health of a honey bee colony can influence the foraging productivity and therefore impact the monetary value of beekeeping, particularly for large-scale commercial apiaries. Honey bees are a billion dollar industry, with most of the revenue derived from the pollination of crops and transgenic seeds. However, honey production still presents a lucrative opportunity for business, illustrated by the $210 million generated from Canadian honey in 2015 [10].

Our objective is to develop a phenomenological mathematical model that can shed light on the impact of *N. ceranae* on colony performance and honey production. We will base this on established tools of mathematical ecology and mathematical epidemiology. The model that we develop will extend and combine a previous model for food and population dynamics in uninfected honeybee colonies that has been introduced in [11], and a previous model of nosemosis that was proposed in [12]. One focus of our study will be on the role of supplementary sugar that is supplied by beekeepers to sustain the colony during winter.

Apis mellifera. The western honey bee, *Apis mellifera*, is a complex eusocial insect forming colonies whose adult females population can peak in the high tens-of-thousands. The most important bee in the colony is the queen, whose sole purpose is to lay eggs that develop into either male drones or female workers. In a single colony, there typically exists only one queen, several hundred male drones and thousands of female workers [13]. Under optimal conditions, a queen may live a number of years, whereas workers and drones have significantly shorter lives. During the winter, adult worker bees may live up to around 154 days [14]. However, during spring, summer and fall, when the workers are forag-

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ing for nectar and pollen, they may only survive 2-3 weeks due to the substantial stress caused by foraging flights [13].

In addition to sex-specific roles in a colony, females are further subjected to an age-based division of labour, referred to as 'temporal polyethism'. The typical schedule of a female worker's duties is as follows: cell cleaning (1-4 days old), nursing duties (4-12 days old), hive maintenance and food storage (12-21 days old), and foraging (>21 days old) [15]. This schedule is regulated through pheromones ethyl oleate (EO) and juvenile hormone (JH) and can be accelerated or decelerated based on food stores or the ratio of hive to forager bees [16, 17]. We will refer to any adult bee that is not foraging as a 'hive bee' and otherwise as a 'forager bee'.

In temperate regions, honey bees are seasonal insects, whose behaviour and biology change depending on variations in ambient temperature throughout the course of a year. Honey bees most notably change behaviour during the winter season as the primary concern of the colony is to maintain an inner hive temperature that allows the colony to survive. During the winter, the queen no longer lays eggs, and forager bees abandon their duties and return to the hive to focus on generating heat to sustain the colony through winter. Hive bees no longer clean cells or tend to brood since cells are no longer needed for eggs or incoming foraged goods. Despite temperatures dropping below -30^{o}C in Canada, the internal temperature of honey bee colony remains at approximately 21^{o}C [18]. The maintenance of inner colony temperature requires a tremendous amount of energy, which, therefore, requires increased honey or sugar consumption. If honey or sugar stores are depleted during the winter, a colony is not likely to survive until spring.

Nosema ceranae biology. The microsporidium *N. ceranae* is an obligate intracellular spore-forming parasite, typically requiring epithelial cells of the honey bee ventriculus to proliferate [19]. After ingestion, spores undergo germination followed by the extrusion of their polar tubes which is used to transfer sporoplasm from the spore into the cytoplasm of the ventricular epithelial cell. The immature spores develop in this epithelial cell for approximately one week before the cell bursts and sheds the new mature spores into the ventriculus at which point they can either infect other cells or can exit the bee through defecation [20]. The spores are able to survive outside the host, although some may lose their ability to infect honey bees, referred to as the spore's 'viability' [21]. *N. ceranae* spores have been observed to maintain viability under natural hive temperatures at approximately 35^{o}C [22]. Visible infection can be observed under a microscope as early as two days post inoculation (dpi) and as late as six dpi [23, 24, 25]. The degree to which parasite reproduction is optimized depends on nutritional availability, initial dosage of spore inoculum and time post inoculation [21, 25, 26]. Although it is hypothesized that spore proliferation eventually reaches a carrying capacity, the timing and degree of infection at which this occurs is debated. While one study observed spore counts to increase from 13 dpi until 19 dpi, at which point all bees died [27], others observed a plateau in spore proliferation to occur between 12 and 15 dpi [25, 28, 29, 30]. The average infection level at this plateau is highly variable from 10 to 25 million spores per bee between studies [25, 30]. Furthermore, the hypothesis of a plateau is contradicted by the extreme variability in spore counts in individual bees [6, 31, 32] and composite samples [8, 33]. It is possible that the plateau of spore proliferation occurs along the same timeline, but the level of infection at which this occurs is different between individual bees, colonies, apiaries, or geographical regions of the world.

It is widely agreed that infection with *N. ceranae* predominantly occurs in the ventriculus, i.e., the mid-gut, of the honey bee, which causes degeneration of the epithelial cells lining the gut [19, 24, 27, 34, 35, 36, 37]. Infection of the intestinal tract supports the hypothesis of transmission via feces [20] and that one of the major effects of infection is increased signs of hunger and food consumption [15, 38, 39].

Effects of N. ceranae. on honey bees. Perhaps the most controversial pathological repercussion of *N. ceranae* infection is whether or not it is associated with increased mortality and if so, to what degree. Two field studies documented increased mortality in *N. ceranae* infected bees. One observed the average lifespan to decrease from between 28-54 days (uninfected control bees) to between 16-23 days (infected bees) over multiple trials [40], while another study observed a significant increase in mortality after 15 days [41]. This trend was also observed in a third study in which mortality rate increased by a factor of 1.99 in infected bees after 16 days post-eclosion, roughly when bees would begin foraging duties [42]. Conversely, one observational field study was not able to find significant colony-level differences in mortality between individual infected and uninfected bees over a five year period [43]. With respect to cage studies, there is much more evidence supporting infection-induced mortality, but results remain inconsistent across the literature. This inconsistency is exemplified by the studies that did observe increases in mortality [23, 27, 29, 34, 42, 44, 45, 46, 47, 48, 49, 50] and those that did not [15, 28, 51, 52, 53, 54]. To add further uncertainty, *N. ceranae*-induced mortality has been observed to be inconsistent through multiple trials in a single study. Under the same conditions in three separate trials, [51] observed the median survival time for infected bees to be 23, 7 and 19 days. Additionally, mortality is believed to be associated with the amount and quality of food consumption, which may confound field studies that were performed in areas with abundant, nutrient-rich types of pollen or nectar [25].

There is evidence to support the hypothesis that *N. ceranae* may induce accelerated progression of polyethism, leading to premature foraging [15]. In most studies in which it was measured, ethyl oleate (EO) was seen to significantly increase in *N. ceranae*-infected bees, in one study being approximately 7 times higher than in uninfected bees, suggesting a possible mechanism for infection-induced accelerated polyethism [40, 55]. Furthermore, these studies also observed positive correlations between EO levels and spore loads. Juvenile hormone (JH) and octopamine concentrations, both of which are associated with foraging behaviour, were higher in infected bees than uninfected bees, further suggesting an earlier onset of foraging [37, 56]. The effects of JH and octopamine on earlier foraging, however, are debated [42]. Infected bees perform behaviours consistent with a faster progression through polyethism. This includes nearly twice as many foragers in an infected group versus a control group [42], decreased standing and increased walking at approximately 12 dpi [15, 52], increased hunger [38], and an earlier onset of food-related communicative dancing [15].

It is possible that flight behaviour is altered in bees infected with *N. ceranae*. While total flight time was highly variable between studies, it is unclear whether or not *N. ceranae* infection increases the time it takes for bees to return to their hive. In one case, there was no difference in homing time between infected and uninfected bees [32], but in another study, infected bees spent twice as long returning to the hive than uninfected bees [57]. The latter observation of prolonged foraging is theoretically supported through increased genetic changes of Octß2R expression, a neurohormone receptor associated with increased foraging time [37].

The hypothesized energetic stress induced by *N. ceranae* infection can be supported by the observation that infected bees are more responsive to and consume more sucrose than their uninfected counter-parts. This is true for cage studies [38, 50, 58, 59], but also for free-flying forager bees in which trehalose levels in infected bees were significantly lower and were depleted quicker than in uninfected foragers [60]. The increased hunger, subsequently leading to an increased rate of starvation in infected bees [38], is perhaps due to an increased metabolism of sugars in the midgut of infected bees [34]. This increased metabolic requirement can be amplified in cold and windy weather in which the bees would need to work harder to fly and to keep a consistent core temperature [38].

Seasonality, transmission and treatment. The transmission of *N. ceranae* plays a particularly important role in understanding disease dynamics and is critical in the formulation of a mathematical model.

Those who observe seasonality of *N. ceranae* typically observe the peak or trough in prevalence to occur during the winter or spring, respectively, although there is disagreement as to whether or not *N. ceranae* infection is truly seasonal. During the winter months, studies have observed a trough in prevalence [9], significant differences between months (November-March) [9], and peaks in infection intensity in individuals bees [33, 61], which may be due to bees being unable to leave the hive to defecate [33]. For the spring months, studies have observed a peak in prevalence [6, 31, 62], and decreasing prevalence, although never reaching a trough [6, 61, 63]. The summer and fall seasons were largely variable such that prevalence and intensity of the disease were observed to peak in some years and yet be on a decline in others [6, 62].

Queen bees are susceptible to horizontal transmission from both workers [48] and male drones whose semen can be naturally contaminated with viable *N. ceranae* spores and can infect queens following insemination [64]. However, there is no evidence that queen bees can transmit infection to their eggs and subsequently pupae, also referred to as vertical transmission [64]. Whether or not transmission can occur horizontally between workers is a debated topic. The mouth parts of bees can be infected with a few thousand spores, likely via cell cleaning [65], and may play a role in the transmission of infection through trophallaxis. This transmission route, however, is inconclusive as the cage study in which the hypothesis was investigated was confounded through potential environmental contamination [66].

The generally accepted hypothesis of disease transmission is a feco-oral route by which infected bees deposit spores into the environment that are later ingested by other bees [67]. A healthy bee ingesting a sufficient number of spores will become infected with *N. ceranae*, at which point they may begin contributing to the environmental reservoir of available spores. Since the ventriculus, being part of the intestinal tract, is the tissue in which spores are most abundant, it seems reasonable to suggest that the primary way in which spores are made available to other bees would occur through defecation. This is observed empirically in [65] in which wall rinsate samples from a cage of infected honey bees were positive for *N. ceranae*. Furthermore, transmission of the related microsporidian, *Nosema apis*, was also observed to be through the feco-oral route [68].

For some time, the only antimicrobial treatment for *N. ceranae* was fumagillin, marketed and sold as Fumagilin-B by Medivet Pharmaceuticals Limited based in Alberta, Canada. Due to the cessation in production of the active ingredient, fumagilline dicyclohexylamine, the company was no longer able to produce fumagillin. Section 4 will introduce a change to our model to represent treatment with fumagillin. In these simulations, we suggest that fumagillin is used as merely an example since there is literature we can draw from to parameterize our model. While we acknowledge that these results are limited, this section will investigate the potential benefits of treatment in general.

Mathematical models. Combining the techniques and approaches of mathematical epidemiology and theoretical ecology, several models of disease dynamics in honeybee populations have been proposed, either for specific or generic diseases. Many of these focus on varroatosis, cf the papers discussed in the review article [69] or some newer models in [70, 71, 72]. Other models focus on viral and other contagious diseases, some of which are vectored by *Varroa destructor* [73, 74, 75, 76, 77].

Most of these dynamic models are studied in an autonomous setting, i.e. it is assumed that model parameters are constant. This allows the application of the relatively well developed methods of non-linear dynamics to study these models. Models that explicitly account for seasonality in population and disease dynamics include [78, 79].

Mathematical models of the spread of a *N. ceranae* infection in a honey bee colony have been introduced in [12, 80]. Both models distinguish between hive and forager bees, based on a honeybee population model that was originally introduced in [81]. Accounting for the disease required a further distinction between healthy and infected worker bees, i.e., a total of four sub-populations are considered. This is in contrast to models of other bee diseases where infected hive bees quickly die and do not become foragers, cf the Acute Bee Paralysis model of [76].

The SIR model presented in [80] implements a direct transmission route and is modelled with mass action kinetics, i.e., a linear force of infection. In contrast, the model in [12] implements an indirect transmission route in which infected bees deposit spores in the environment and are acquired by hive bees during hive cleaning. A further distinction between [80] and [12] is how seasonality effects are incorporated. Both models recognise the importance of the population dynamics during winter for the long-term fate of the colony. In [80] two seasons are considered: a single winter season, and an 'active season'. In each season, the model parameters are constant and the long-term dynamics in the last season are studied. In [12], the year is divided into four equally long seasons: spring, summer, fall, and winter, which repeat themselves from year to year, resulting in a non-autonomous model with time-periodic parameters. This approach was adapted from [76, 79], who modelled the spread of *Varroa* mites in a colony of honey bees. In [12], removal of spores from the hive by the beekeeper as part of maintenance efforts is included as a remedial strategy. An extension of the model of [12] to study between-hive transmission of *N. ceranae* among neighbouring colonies is presented in [82]. In [83], a model is introduced that combines aspects of the models of [12] and [80] by considering both routes of transmission, direct and indirect.

Neither of [12, 80] nor the models that build on them consider the effect of the disease on the colony's honey production, nor do they account for the change in nutritional requirements of a honey bee when it becomes infected with *N. ceranae*. An extension of the honey bee population model [81], on which the *N. ceranae* models of [12, 80] are based, was presented to account for such questions in the absence of disease in [11].

Objective. The microsporidian *N. ceranae* is an important parasite to the health of honey bees, yet remains poorly understood. Discrepancies between studies, or even within different trials of the same study, have not allowed for a comprehensive understanding of the pathogen and therefore its effect on honey bees at the colony level. These impediments are not only due to uncertainty of the pathogen, but also the difficulty in obtaining reliable results from studying populations of honey bees *in vivo* or *in situ*. While cage studies can be useful for minimizing confounding variables, results often have limited external validity due to unrealistic conditions such as environment, handling stress, access to food and the absence of a queen or brood. However, otherwise it would not be possible to obtain these results. Conversely, field studies present the inability to control for certain confounding variables and can be expensive and labour intensive to execute properly. By using relevant assumptions and appropriate structure, a mathematical model can be used to study the effect of *N. ceranae* in a colony of honey bees. Based on the background literature presented above, we believe that we have provided sufficient information on *Apis mellifera*, *Nosema ceranae*, and existing honey bee models to suggest contributions that can be made to further develop an understanding of the effects of *N. ceranae* through mathematical modelling. Currently, the only *N. ceranae*-specific mathematical model that accounts for the primary, indirect transmission is presented by [12]. This model, however, does not consider the dynamics of food stores. Not only is the basic biology of honey bees heavily dependent on food, the main effects of *N. ceranae* are also predominantly food-based. This includes a reduced foraging efficiency [3, 32, 41, 57], increased food consumption [34, 38, 50, 58, 59, 60] and differential rates of starvation between healthy and infected bees [38]. Additionally, a mathematical model of *N. ceranae* including food dynamics allows for analysis regarding the economic benefits of various treatments or interventions. Using annual and long-term honey yield as a quantity of interest, we can quantify the effects of supplementary sugar feeding and fumagillin treatment. These results would otherwise not be possible to obtain if

one were to use the existing models available in the literature. The model that we will propose also references elements from the population and food dynamics model presented by [11]. However, the model in [11] does not study *N. ceranae*, nor does it include seasonal variation of parameter, nor the effects of honey harvest and supplementary feeding. We therefore suggest that an opportunity exists to study an *N. ceranae* infection in a colony of honey bees by building upon the model frameworks presented by [11] and [12] through the inclusion of food dynamics and seasonal parameter variation.

2. Mathematical model

2.1. Model assumptions

Our model will be based on the following assumptions:

1. We follow the work of [11, 12, 74, 80, 81, 84, 85, 86] and classify adult honey bees as either hive bees or forager bees. Hive and forager bees are further sub-divided into healthy and infected bees similar to the structure presented in [12, 76, 80, 84, 87].
2. Similar to the *Nosema* work of [12, 80, 82, 83], and other disease models in [73, 74, 76, 77, 79] we assume the queen and drone bees are not affected by *N. ceranae* and will not be infected. The maximum emergence rate is therefore affected only by the natural seasonal variation of the queen's egg laying rate in addition to a sufficient number of bees to rear brood, but not the health of the queen.
3. *N. ceranae* is not transmitted vertically [64], therefore all bees that emerge do so as healthy hive bees.
4. To reduce the complexity of our model, the brood is not explicitly considered, as in [12], in accordance with other bee disease models [74, 76, 79, 80, 84]. Since vertical transmission cannot occur and brood do not partake in cell cleaning, it is not possible for brood to become infected and, therefore, they do not play a role in disease transmission. We do, however, consider implicitly the role of brood when accounting for the emergence of bees and the amount of food consumed by brood over the duration of their maturation into adult worker bees.
5. We do not consider the delay between egg laying by the queen and subsequent emergence of mature adult worker bees, also in accordance with other bee disease modelling studies [74, 76, 77, 79, 80, 84].
6. Similar to the assumption above, we do not consider a delay between the foraging of pollen and nectar and its subsequent processing into honey. Although this is not explicitly stated there, this assumption follows the work of [11, 80, 86, 88].
7. Following [11], we do not differentiate between pollen, nectar and honey as food sources, but rather refer to all as "food". However, we will refer to harvested food as honey.
8. Since the foraging efficiency of infected bees is compromised [3, 32, 41, 57], we differentiate between the amount of food collected by infected and uninfected foragers.
9. As the amount of stored food per bee is reduced below the minimum amount of required food per bee, bees will begin die due to starvation.
10. Hive and forager bees have similar rates of food consumption [11], however, infected bees will consume more food than uninfected bees due to the energetic stress of infection [34, 38, 50, 58, 59, 60].
11. We assume that bees consume food at a close to constant rate as long as it is available, but food consumption decreases proportionally when it becomes limited.
12. Honey bees will preferentially feed on sugar syrup when it is available [89].
13. Honey bees consume more food during the winter. This is an *ad hoc* assumption that we introduce here. There seem to be no quantitative data in the literature, likely due to the challenges for experimental design. While it is possible to conduct calorimetric measurements during Winter, non-disruptive measurements in Spring/Summer are more difficult to envision, in particular since also consumption during foraging would need to be accounted for. Our assumption here is that in Northern climates a colder winter requires bees to consume more food to have the energy to maintain a consistent inner-hive temperature. In the absence of sufficient data, we will assume bees consume 10% more food during the winter.
14. The daily amount of food needed before starvation occurs in infected bees is twice that of uninfected bees [38].
15. The only route of transmission considered in this model is indirect through ingestion of viable spores picked up during cell cleaning [12]. Spores are deposited into the environment by infected hive bees through defecation [20] and can either be ingested by hive bees during hive cleaning or lose viability over time [21]. As in [12], the environmental reservoir of viable spores will be referred to as the 'environmental potential'.
16. Following the work presented by [12, 77, 79, 83, 90], many parameters are time dependent and will fluctuate between spring, summer, fall and winter.
17. Honey is harvested from hives once a year on the last day of fall. The amount of honey harvested depends on the amount of honey collected by bees such that at the beginning of winter, approximately 100g of honey remains in the hive.
18. The only disease or ailment considered in this model is the microsporidia *Nosema ceranae*, allowing us to refer to "uninfected" or "healthy" bees synonymously.
19. Recruitment of bees to foraging duties accelerates when the amount of food per bee stored in the hive becomes smaller than the daily food requirement. This assumption is a slight variation of the assumption presented in [11, 86].
20. As in [12], the death rates of hive bees in the spring, summer and fall months are negligible compared to the rate at which they are recruited to foraging duties.

Assumptions 1, 2, 3, 4, 15, 16, and 20 are referenced from [12], and assumptions 1, 6, 7, and 19 and referenced from [11], assumptions 8, 9, 10, 11, 12, 13, 14, and 17 are introduced in this paper. A compartmental diagram that summarizes the processes that are considered in the model is given in Fig. 1.

2.2. Governing equations

In our system of ordinary differential equations, we compartmentalize bees based on their worker and infection status: healthy hive bee (H_0), infected hive bee (H_1), healthy foraging bee (F_0), or infected foraging bee (F_1). E represents the environmental reservoir of *N. ceranae* spores which we refer to as the environmental potential, as in [12]. Finally, we model food stores by differentiating between honey (f) and supplementary sugar syrup (s). The system of equations governing the model is as follows:

$$\dot{H}_0 = \underbrace{\tilde{\beta}(Z,t)}_{Emergence} + \underbrace{\sigma\frac{F}{Z}F_0}_{Reversion} - \underbrace{R(Z,f,s,t)H_0}_{Recruitment} - \underbrace{\tilde{\eta_0}(Z,f,s,t)H_0}_{H_0\,death} - \underbrace{\alpha(t)\frac{E}{\lambda+E}H_0}_{Rate\,of\,infection} \tag{1}$$

$$\dot{H}_1 = \underbrace{\alpha(t)\frac{E}{\lambda+E}H_0}_{Rate\,of\,infection} + \underbrace{\sigma\frac{F}{Z}F_1}_{Reversion} - \underbrace{R(Z,f,s,t)H_1}_{Recruitment} - \underbrace{\tilde{\eta_1}(Z,f,s,t)H_1}_{H_1\,death} \tag{2}$$

$$\dot{F}_0 = \underbrace{R(Z,f,s,t)H_0}_{Recruitment} - \underbrace{\sigma\frac{F}{Z}F_0}_{Reversion} - \underbrace{\tilde{\phi_0}(Z,f,s,t)F_0}_{F_0\,death} \tag{3}$$

$$\dot{F}_1 = \underbrace{R(Z,f,s,t)H_1}_{Recruitment} - \underbrace{\sigma\frac{F}{Z}F_1}_{Reversion} - \underbrace{\tilde{\phi_1}(Z,f,s,t)F_1}_{F_1\,death} \tag{4}$$

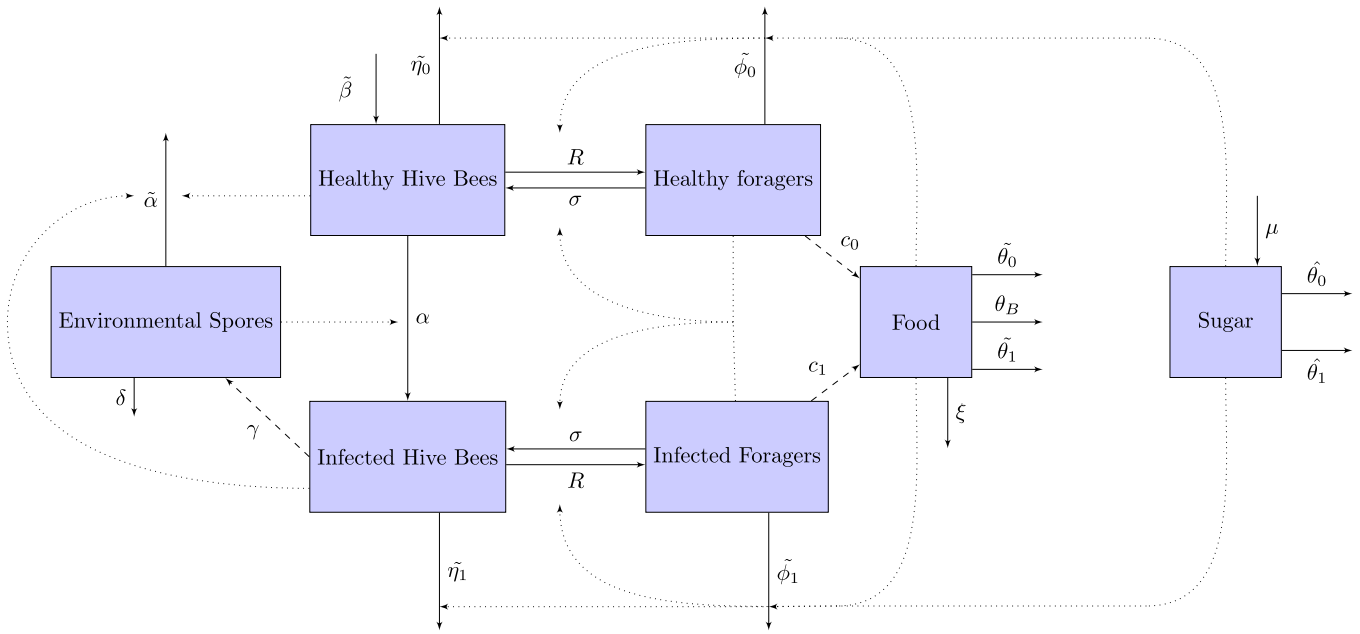


Fig. 1. Compartmental diagram for the model - parameters and functions will be explained in detail in Section 2.2. Each compartment represents an equation of the model with the parameters or functions included to present a visual representation of how they are used. The solid lines represent physical transitions of the compartments, for example: food being removed from the f compartment via consumption at rates θ_0, θ_1, and θ_b. The dashed lines represent a contribution from one compartment to another, for example: spores deposited into the environment from infected hive bees at rate γ. Finally, the dotted lines represent an influence of one compartment on the transition (solid lines) to another compartment, for example: the amount of food and supplementary sugar influence the death rates of bees in addition to influencing the rate at which bees are recruited to foraging duties. Note that all θ_0 and θ_1 terms are influenced by the number of healthy and infected bees, respectively, but are omitted for the sake of clarity.

$$\dot{E} = \underbrace{\gamma(t)H_1}_{\text{Deposition of spores}} - \underbrace{\delta(t)E}_{\text{Loss of viability}} - \underbrace{\tilde{\alpha}(t)\frac{E}{\lambda+E}H}_{\text{Removal of spores}} \tag{5}$$

$$\dot{f} = \underbrace{c_0(t)F_0 + c_1(t)F_1}_{\text{Food collection}} \underbrace{-\tilde{\theta}_0(f,s,t)(H_0+F_0) - \tilde{\theta}_1(f,s,t)(H_1+F_1)}_{\text{Food consumption (workers)}} - \underbrace{\theta_B(Z,t)}_{\text{Food consumption (brood)}} \underbrace{-\xi(f,t)f}_{\text{Harvest}} \tag{6}$$

$$\dot{s} = \underbrace{\mu(t)}_{\text{Sugar addition}} \underbrace{-\hat{\theta}_0(s,t)(H_0+F_0) - \hat{\theta}_1(s,t)(H_1+F_1)}_{\text{Sugar consumption}} \tag{7}$$

where we use the shorthand notation $H := H_0 + H_1$ (i.e., the total number of hive bees), $F := F_0 + F_1$ (i.e., the total number of forager bees), and $Z := H_0 + H_1 + F_0 + F_1$ (i.e., the total number of bees).

The named functions presented in the system of ordinary differential equations are defined below. Parameters and coefficient functions have the following meaning:

Nutritional requirement. We introduce the parameters a and b which represent the minimum amount of food required per healthy or infected bee, respectively, for a colony to function under 'normal' circumstances of food abundance. As the amount of food per bee is reduced below either a for healthy bees, or b for infected bees, the rates at which bees die and are recruited from hive to foraging duties are increased. The value of a is set to 4 mg/bee, which was determined to be the minimum amount of daily utilizable sugars required for bees to survive [91]. Based on our assumption that *N. ceranae* induces energetic stress, causing infected bees to consume twice as much food, we set b to 8 mg/bee.

Emergence of adult bees. The function $\tilde{\beta}(Z,t)$ represents the maximum emergence rate of healthy hive bees, which is a product of the queen's egg laying rate, $\beta(t)$, and the brood maintenance function from [90] with half-saturation constant $k(t)$,

$$\tilde{\beta}(Z,t) = \beta(t)\frac{Z^2}{k(t)^2+Z^2} \tag{8}$$

While we assume that the egg-laying rate of the queen is a given periodic function, the success of brood emergence depends on the number of adult bees in a colony: If the number of workers in the colony drops significantly below $k(t)$, the brood cannot be taken care of sufficiently.

Recruitment to foraging duties. Worker bees belong to one of two classes: hive bees or forager bees. The rate at which bees transition from hive to foraging duties is given by $R(Z,f,s,t)$. Similar to [11], it consists of a baseline recruitment rate, ψ_{min}, and an additional recruitment rate, ψ_{max}, that depends on food availability in the hive. We modify the function in [11] such that it also depends on the minimum daily food requirement, a. We have:

$$R(Z,f,s,t) = \psi_{min}(t) + \left(\psi_{max}(t)\frac{a^2}{a^2+(\frac{f+s}{Z})^2}\right). \tag{9}$$

Death rates. The death rates of adult bees vary between hive bees and foragers, and between uninfected and infected bees. Each class has its own baseline death rate (η_0, ϕ_0, η_1, ϕ_1). Additionally we account for terms that represent starvation-induced death ($\hat{\eta_0}, \hat{\eta_1}, \hat{\phi_0}, \hat{\phi_1}$) if the amount of food available in the colony per bee drops below daily food requirement parameters. The death rates for healthy and infected hive bees are:

$$\tilde{\eta_0}(Z,f,s,t) = \eta_0(t) + \left(\hat{\eta_0}(t)\frac{a^2}{a^2+(\frac{f+s}{Z})^2}\right) \tag{10}$$

$$\tilde{\eta_1}(Z,f,s,t) = \eta_1(t) + \left(\hat{\eta_1}(t)\frac{b^2}{b^2+(\frac{f+s}{Z})^2}\right), \tag{11}$$

and for forager bees:

$$\tilde{\phi_0}(Z,f,s,t) = \phi_0(t) + \left(\hat{\phi_0}(t)\frac{a^2}{a^2+(\frac{f+s}{Z})^2}\right) \tag{12}$$

$$\tilde{\phi_1}(Z,f,s,t) = \phi_1(t) + \left(\hat{\phi_1}(t)\frac{b^2}{b^2+(\frac{f+s}{Z})^2}\right) \tag{13}$$

A starvation-induced death rate is included in [88], although this may be over-simplified: once food stores reach ≤ 100 grams, the death rate is

set to one, i.e., all bees begin to die. In our model, we derive the value of the starvation-induced death rate from a cage study performed in [92], where it was observed that bees deprived of food lasted an average of 35.6 hours. We therefore choose the starvation-induced death rate such that when added to the baseline death rate the total death rate is $\frac{1}{35.6h} = \frac{1}{1.4833d} = 0.6742/d$.

The parameter η_0 is set to 0 during the spring, summer and fall months since the rate at which healthy hive bees would die is negligible compared to the rate at which they transition into foraging bees. We use data from [93] who observed that under field conditions, the foraging life of European honey bees was just over 11 and a half days, therefore we use $\phi_0 = 0.08511/d$. During the winter, $\phi_0 = \phi_1 = 0$ since forager bees transition back to hive duties and no foraging occurs during the winter. We refer to the paper [42] who examined differences in mortality between healthy bees and those infected with *N. ceranae*. While there were negligible differences in mortality between the two groups for the first 14 days, survivorship of those infected with *N. ceranae* was significantly lower between days 16 and 25 of the study, with an increase in mortality rate by a factor of 1.99. 16 days post-emergence is approximately the time at which bees begin foraging. We therefore do not differentiate between the death rates of hive bees, η_0 and η_1, but suggest instead an increase in death rate between uninfected and infected forager bees such that $\phi_1 = 1.99\phi_0$.

N. ceranae related parameters. The parameters describing disease dynamics are adopted from [12]. The rate at which bees acquire the infection depends on the amount of spores, E, present in the hive. If $E \gg \lambda$, the scaling parameter, the rate of infection approaches it maximum α. If the inequality is reversed, the rate of infection is proportional to the spore levels. $\tilde{\alpha}$ is the rate at which *N. ceranae* spores are removed from the environment by hive bees through cell cleaning. It is assumed to be constant in the spring, summer, and fall seasons but is zero during the winter since hive cleaning does not occur during this time.

N. ceranae spores typically infect and proliferate in the epithelial cells lining the intestines of honey bees. When spores accumulate to the point that an epithelial cell is full and bursts, the mature spores are released into the intestines and can either infect new cells or can be shed from the bee into the environment through defecation. The rate at which these spores are shed through defecation into the hive is represented by γ. While bees typically defecate outside of the hive, temperature and weather can restrict bees from leaving which may lead to pathogen shedding in the hive. Whatever this deposition rate may be, it would reach its maximum potential during the winter when bees are unable to leave the hive. We refer to the winter deposition rate as γ_w and assume that the seasonal values of γ are proportional to the value of γ_w. These values are based on the number of days bees were restricted to the hive due to inclement weather or temperature and based on meteorological data from 2015 [12]. Spores that have been deposited into the environment lose viability at rate δ.

Food collection. Healthy and infected foragers will collect food at rates c_0 and c_1, respectively. The parameter values for healthy forager food collection are taken from [88], who normalized empirical data to parameterize monthly food collection. Since our model uses seasonal parameter values, we take the average of values between March-May, June-August, September-November for spring, summer, and fall, respectively. The only exception was during the winter season (December-February), during which we set the food collection parameters to zero. An important consideration in the food collection parameters are differences between infected and uninfected bees. We refer to the observations that infected bees took 2.1 times as long to return to the hive compared to uninfected bees [57] and can therefore set the infected forager collection parameter as $c_1 = \frac{c_0}{2.1}$. On the day of honey harvest c_0 and c_1 are set to zero. We argue that the vast influx of supplementary sugar replaces the need for bees to forage.

Food consumption. Healthy and infected bees will consume food and sugar at rates θ_0 and θ_1, respectively. Based on the observations in [50, 58] in which caged *N. ceranae*-infected bees consumed approximately twice as much sucrose solution than uninfected control bees, we set $\theta_1 = 2\theta_0$. During the winter, honey bees will aggressively vibrate their bodies to produce thermal energy needed to heat the hive. This process is energetically demanding and requires a large amount of food energy to perform. If winters are particularly cold, more thermal energy is needed to maintain a consistent internal hive temperature and therefore requires greater food consumption. We introduce a 'winter harshness factor', ω, that determines the percent increase in food that a honey bee needs to consume during the winter. We suggest that bees will preferentially feed on supplementary sugar over stored honey as long as it is available [89]. We assume that the affinity for supplementary sugar is twice that of honey, represented by the parameter ν. With this in mind, the food consumption rates for healthy bees ($\tilde{\theta_0}(f,s,t)$) and infected bees ($\tilde{\theta_1}(f,s,t)$) are

$$\tilde{\theta_0}(f,s,t) = \theta_0(t)\frac{f}{h+f+\nu s} \tag{14}$$

$$\tilde{\theta_1}(f,s,t) = \theta_1(t)\frac{f}{h+f+\nu s} \tag{15}$$

Sugar consumption for healthy bees ($\hat{\theta_0}(s,t)$) and infected bees ($\hat{\theta_1}(s,t)$):

$$\hat{\theta_0}(s,t) = \theta_0(t)\frac{s}{h+s} \tag{16}$$

$$\hat{\theta_1}(s,t) = \theta_1(t)\frac{s}{h+s} \tag{17}$$

Note that to maintain positivity of solutions, we use h as a scaling parameter set to an arbitrarily small value. This parameter represents the amount of food in the hive that is necessary for bees to reduce the rate at which they consume food. So long as there is sufficient sugar or honey present in the hive, bees will consume food at their maximum rate, θ_0 or θ_1. Although we do not consider brood with respect to disease dynamics in this model, we account for the amount of food consumed per brood from eclosion to emergence, $\theta_B(Z,t)$. This function is represented by the product of θ_b, the average amount of food consumed by brood for the duration of their pupation and $\tilde{\beta}$, the number of bees that emerge as adult bees. We use

$$\theta_B(Z,t) = \theta_b(t)\beta(t)\frac{Z^2}{k(t)^2+Z^2}. \tag{18}$$

Honey harvest and supplementary sugar addition. We assume that harvesting of honey and addition of supplementary sugar by the beekeeper are carried out once per year over a short time span of duration $t_h - t_0$, starting at time t_0 at the end of fall. The rate of honey harvesting depends on both $f(t_0)$ and the target amount of food that will be left in the hive after harvesting. Assuming a constant rate of removal of the harvesting period, we calculate the harvesting rate to be

$$\xi = \frac{-\ln\left(\frac{f(t_h)}{f(t_0)}\right)}{t_h - t_0}. \tag{19}$$

Thus the amount of honey harvested each year depends on the food stores. We assume that over the same time interval, additional food may be added to the hive in form of 2:1 sugar syrup, as a means to sustain the colony through the winter. This parameter can change based on the manner of which the sugar is added to the hive, but will mostly be kept at 13,000 grams to represent a fully filled top-feeder frame.

Time dependency of model parameters. As per assumption 16, most model parameters are time dependent. Values in the literature, such as [77], are often reported as seasonal averages. This is the structure that we adopt. We assume that each seasons season consists of 91 days, except fall which has an extra day for the honey harvest. Throughout each season we keep the parameters constant, as in [90]. Other studies have argued that it might be more reasonable to use continuously varying parameters instead. However, the generalisation from seasonally averaged to continuous functions is possible in many different ways,

each of which would introduce additional model parameters and degrees of freedom. See [12] where this has been done for Nosemosis and [79] for the Acute Bee Paralysis Virus. While the choice of these additional parameters may effect quantitative predictions, it was found that the impact on qualitative behaviour is minimal. Therefore, in order to avoid introducing additional degrees of freedom we use here piecewise constant functions. Due to the discontinuity of model parameters, we then understand model (1)-(7) as differential equations in the sense of Caratheodory; existence, uniqueness, positivity and boundedness of solutions almost everywhere can be established in a straightforward manner with standard arguments [94, 95].

The parameters used in our simulations are summarized in Table 1.

2.3. Computational setup

Equations (1)-(7) are an extension of the model in [12], which already did not lend itself to much insightful mathematical analysis. Therefore we conduct a numerical exploration. All simulations were carried out with the software `R` [96], using the ordinary differential equation solver package `deSolve` [97] to numerically integrate the system of equations. Simulations begin on the first day of spring with initial populations $H_0(1) = 5,000$ and $F_0(1) = 2,500$ and initial sugar $s(1) = 500$. Food has not yet been foraged, so $f(1) = 0$. We also assume that initially disease is absent, $H_1(1) = F_1(1) = E(1) = 0$. An initial period of one year is simulated to allow the system to equilibriate. When studying the dynamics of the disease, we alter the populations such that ten healthy hive bees become infected on the first day of winter during the first year, i.e., $H_0(275) = H_0(274) - 10$ and $H_1(275) = 10$, with $t = 275$ being the first day of winter of the first year. We restrict the scope of our results and observations to within 10 years of the beginning of the colonization. We argue that this is already an unusually long time for a colony to last without either failing, swarming or being combined with another colony to increase probability of winter survival. For technical reasons, in order to account for beekeeper intervention for harvesting and external sugar supply, the model is coded so that it is broken into three phases: pre-harvest (spring, summer, and fall at 91 days each), harvest (1 day period between fall and winter), and post-harvest (91 days of winter conditions).

3. Simulation results

3.1. Sensitivity analysis

We performed a sensitivity analysis of the model through Latin Hypercube Sampling (LHS; `R` package `lhs` [98]) and subsequent calculation of the partial rank correlation coefficients (PRCC; `R` package `sensitivity` [99]) for each specified parameter. Our outcomes of interest are the sum of honey harvested over ten years and the number of days before colony failure occurs within a ten year period. We run 1000 iterations of the model under various sets of parameters and use the simulation data in our PRCC.

We test the parameters ϕ_1, θ_1, θ_b, b, as well as the spring, summer and fall values for c_1, α, and δ. These parameters are chosen since, for the most part, they contribute to either food collection, consumption, or the dynamics of *N. ceranae*. We set the bounds on possible parameter values to be ±50% of the values referenced in Table 1. For the parameters $\tilde{\alpha}$, and γ_w, we test the range between 1.0-3.0 for both, which is the range in which they were varied in [12], where they were originally introduced. For ω we only test the range 1.0-1.5, but not values below, since it is our assumption that bees consume more food during the winter than they would during the other seasons.

We first test the partial correlation between those parameters and the 10 year honey yield sum, cf Fig. 2. The results indicate that the model is most sensitive to parameters θ_b, γ_w, and $\tilde{\alpha}$. It is not surprising that the sum honey yield has a strong negative correlation with θ_b, the amount of food consumed over 9 days of brood maturation. The value of θ_b is larger than either the food collection or worker bee consumption parameters, so even small perturbations to this parameter can lead to large changes with respect to long-term honey yield. Parameters $\tilde{\alpha}$ and γ_w both contribute to the disease dynamics as well as having significant positive and negative correlations to the ten year honey yield sum, respectively. We therefore study the model dynamics under various combinations of these parameters.

The second sensitivity analysis tests the correlation between the parameters listed above and the time until colony failure, which we define, somewhat arbitrarily, to be the day at which the sum of bees is less than one, i.e., $H_0 + H_1 + F_0 + F_1 < 1$, see Fig. 3. Of 1000 simulations, 131 parameter sets resulted in colony failure at some point before the end of the 10 year simulation period. This quantity of interest is most sensitive to $\tilde{\alpha}$ and γ_w, and with similar partial correlation values to the previous sensitivity analysis. This further supports our decision to study the model dynamics under various combinations of these parameters.

3.2. Preliminary and preparatory simulations

We summarize here simulations that describe the behaviour of the model with and without disease. This section is included here to show model behaviour and to establish a base line for reference later on. The qualitative aspects of the disease model under consideration here were discussed in more detail previously in [12], on which our extended model is based.

3.2.1. Disease-free model dynamics: base-case simulation

We briefly illustrate the population dynamics of a honey bee colony without disease under standard parameters, shown in Fig. 4. The population of hive bees remains constantly higher than foragers with total populations increasing during the spring and summer and then decreasing during the fall and winter. The total population of bees peaks during the summer with approximately 33,000 hive bees and 15,000 foragers, well within the range that is observed by beekeepers in the field. The trough in population occurs at the end of the winter with approximately 7,800 hive bees and 100 foragers. The small spike in hive population at the beginning of winter is due to foragers reverting back to hive duties.

After approximately two years, the model reaches almost periodic dynamics, where population sizes in subsequent years repeat themselves, i.e., a limit cycle. Since no bees are infected with *N. ceranae*, the environmental potential remains at zero through the entire 10 year simulation. The amount of honey increases through the spring, summer and fall and drops sharply when the honey is harvested on the last day of fall. On the same day, the supplementary sugar is added and gradually decreases as it is consumed. Similar to the bee population, the amount of honey reaches consistent dynamics in the second year with annual honey yields being nearly identical after the first year. We note that the honey yield from the first year is smaller by approximately 7 kg compared to the following years. This is attributed to the smaller initial population of bees at the beginning of the first year compared to the first day of spring in subsequent years. Under the parameters used here, the annual honey yield is approximately 35.1 kg, which is within the range of honey yield reported by the USDA for 2017 (between 14.1 kg in Utah and 59.4 kg in Hawaii, US average: 25.1 kg) [100], and by Statistics Canada for 2017 (between 8.7 kg in Nova Scotia and 86.6 kg in Saskatchewan, Canadian average: 52.9 kg) [101]

3.2.2. Effects of disease on model outcome

Our model is an extension of the one introduced in [12], for which no comprehensive mathematical analysis was possible due to the model's complexity. Therefore we study the effect of spore uptake rate ($\tilde{\alpha}$) and winter spore deposition rate (γ_w), i.e. the parameters that have the strongest influence on the outcome of the solution in simulation studies, keeping all other parameters at their default values. In these simulations the amount of supplementary sugar that is externally supplied is at default values, i.e. high enough that it helps the colony survive the winter.

Table 1. Parameter values used in the simulations. To account for seasonal variations, we consider different parameter values for spring, summer, fall, and winter. The parameters describing honey harvest and sugar feeding are restricted to a period of one day at the end of fall*.

Parameter Name	Symbol	Units	spring	summer	fall	winter	Reference
Maximum emergence rate	β	$bees \cdot d^{-1}$	500	1500	500	0	[12]
Brood maintenance constant	k	$bees$	8000	12000	8000	6000	[12]
Healthy bee min. food requirement	a	$grams \cdot bee^{-1}$	0.004	0.004	0.004	0.004	[91]
Infected bee min. food requirement	b	$grams \cdot bee^{-1}$	0.008	0.008	0.008	0.008	[91], assumed
Rate of reversion	σ	d^{-1}	1.5	1.5	1.5	1.5	[12]
Rate of recruitment (min.)	ψ_{min}	d^{-1}	0.25	0.25	0.25	0	[11]
Rate of recruitment (max.)	ψ_{max}	d^{-1}	0.25	0.25	0.25	0	[11]
H_0 death rate	η_0	d^{-1}	0	0	0	0.00649	[12, 93]
H_0 starvation death rate	$\hat{\eta_0}$	d^{-1}	0.6742	0.6742	0.6742	0.66771	[92]
H_1 death rate	η_1	d^{-1}	0	0	0	0.00649	[12]
H_1 starvation death rate	$\hat{\eta_1}$	d^{-1}	0.6742	0.6742	0.6742	0.66771	[92], calculated
F_0 death rate	ϕ_0	d^{-1}	0.08511	0.08511	0.08511	0	[12]
F_0 starvation death rate	$\hat{\phi_0}$	d^{-1}	0.58909	0.58909	0.58909	0	[92], calculated
F_1 death rate	ϕ_1	d^{-1}	0.16937	0.16937	0.16937	0	[42], calculated
F_1 starvation death rate	$\hat{\phi_1}$	d^{-1}	0.50483	0.50483	0.50483	0	[92], calculated
Infection rate	α	d^{-1}	0.55	0.12	0.24	0	[12]
Spore half-saturation constant	λ	$spores$	10000	10000	10000	10000	[12]
Spore deposition rate	γ	$spores \cdot bee^{-1} \cdot d^{-1}$	$0.2061\gamma_w$	$0.2835\gamma_w$	$0.2527\gamma_w$	γ_w	[12]
winter spore deposition rate	γ_w	$spores \cdot bee^{-1} \cdot d^{-1}$	varied				assumed
Spore decay rate	δ	d^{-1}	0.006570	0.023300	0.015683	0	[12]
Spore uptake rate	$\tilde{\alpha}$	d^{-1}	varied			0	assumed
F_0 food collection rate	c_0	$grams \cdot bee^{-1} \cdot d^{-1}$	0.07933	0.05966	0.034	0	[88]
F_1 food collection rate	c_1	$grams \cdot bee^{-1} \cdot d^{-1}$	0.03778	0.02841	0.01619	0	[88], calculated
Healthy bee food consumption rate	θ_0	$grams \cdot bee^{-1} \cdot d^{-1}$	0.007	0.007	0.007	0.007ω	[11], assumed
Infected bee food consumption rate	θ_1	$grams \cdot bee^{-1} \cdot d^{-1}$	0.014	0.014	0.014	0.014ω	[11, 50, 58], calculated
winter harshness factor	ω	unitless	1	1	1	1.1	assumed
Consumption half-saturation constant	h	$grams$	100	100	100	100	assumed
Sugar preference constant	ν	unitless	2	2	2	2	[89]
Brood food consumption	θ_b	$grams \cdot bee^{-1}$	0.163	0.163	0.163	0	[11]
Honey harvest	ξ	t^{-1}	0	0	varied*	0	calculated
Sugar syrup addition	μ	$grams \cdot d^{-1}$	0	0	13000*	0	assumed

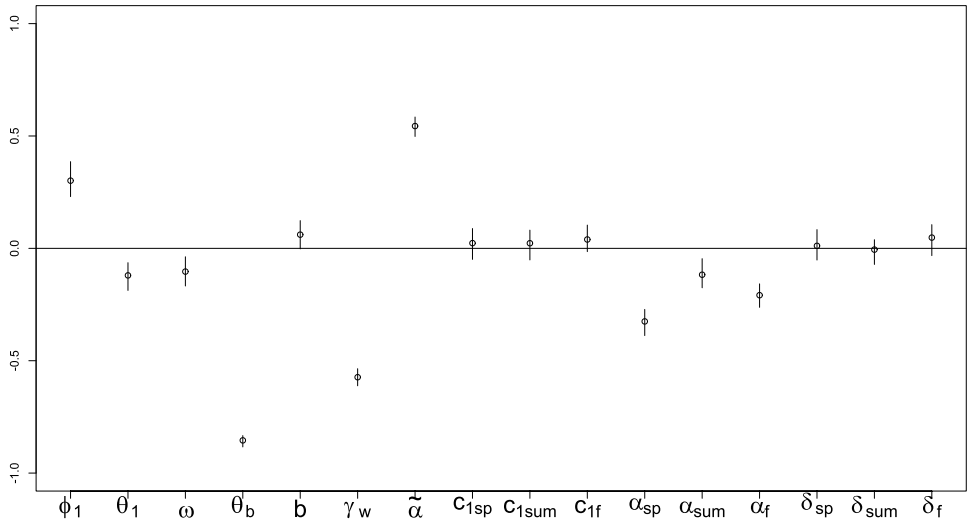


Fig. 2. Sensitivity analysis - partial rank correlation coefficients with 95% confidence intervals for parameters with respect to the total honey yield after 10 years.

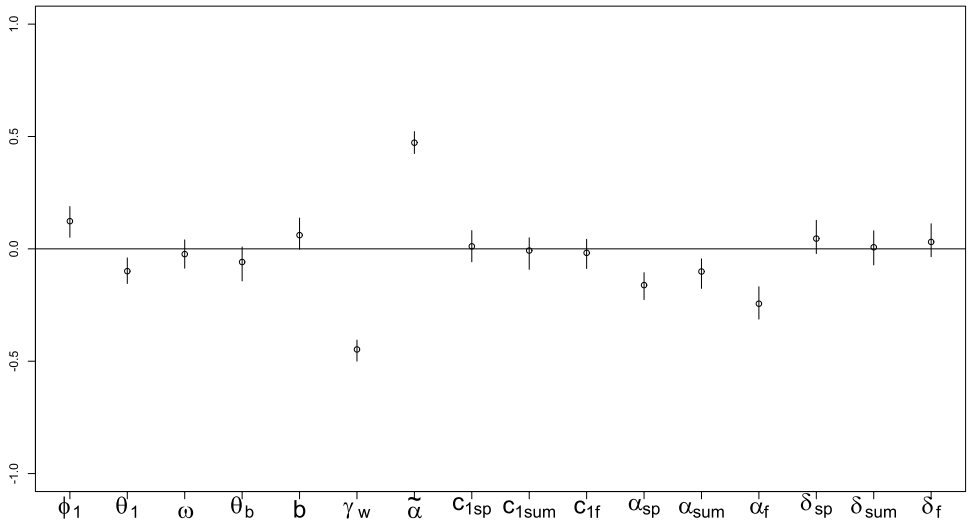


Fig. 3. Sensitivity analysis - partial rank correlation coefficients with 95% confidence intervals for parameters with respect to the number of days before colony failure in a 10 year simulation period.

As previously in [12], three different qualitative model outcomes are observed:

- **colony failure** if the colony population dwindles to zero
- **endemic co-existence** of bees and parasite, albeit at reduced colony strength compared to the disease free case
- **parasite fadeout** if the environmental potential reduces to 0

A depiction of qualitative model outcomes with respect to these two parameters is given in Fig. 5. Noteworthy is that the three regions of model outcome in the $\tilde{\alpha}$-γ_w plane are separated from each other by straight lines. For a given $\tilde{\alpha}$, small values of γ_w will lead to parasite fadeout, and as γ_w passes a first critical value the disease will establish itself in the colony. As γ_w exceeds a second, larger critical value, the colony will fail. The larger $\tilde{\alpha}$ gets, the larger these critical threshold values become.

Nosemosis is a frequently observed disease that is not known to lead to inevitable colony failure, nor is there evidence in the literature that once infected colonies rid themselves of the pathogen. Therefore, the results in Fig. 5 help to narrow down the ranges of parameters $\tilde{\alpha}$ and γ_w to values that lie between or close to the depicted lines. As previously observed in the simulations in [12], our results also show that colony strength decreases in the presence of the pathogen (data not shown). In contrast to [12], we are able to track the amount of honey that is harvested and find that a reduction in colony strength leads to a reduction in honey yield, cf Table 2.

As we might expect, the annual percent honey yield loss increases as the strength of disease increases. Furthermore, in cases of co-existence and colony failure, the percent honey yield reduction increases between years. In the case of disease removal, the annual percent honey yield loss decreases through the years. The colony is able to recover after removal of *N. ceranae* and so both model scenarios eventually produce the same amount of honey each year. Therefore, the percent difference between the two will decrease over time, but will never reach exactly zero.

3.2.3. Colony failure due to starvation

In the previous sections, simulations were carried out for a level of supplementary sugar feeding that is sufficient to completely compensate for honey harvest, at 13000 grams. To explore the effect of supplementary feeding we run several simulations with successively decreased levels of replenishment, see Fig. 6. In the absence of *N. ceranae* we find that when 9600 grams or less of supplementary sugar is added

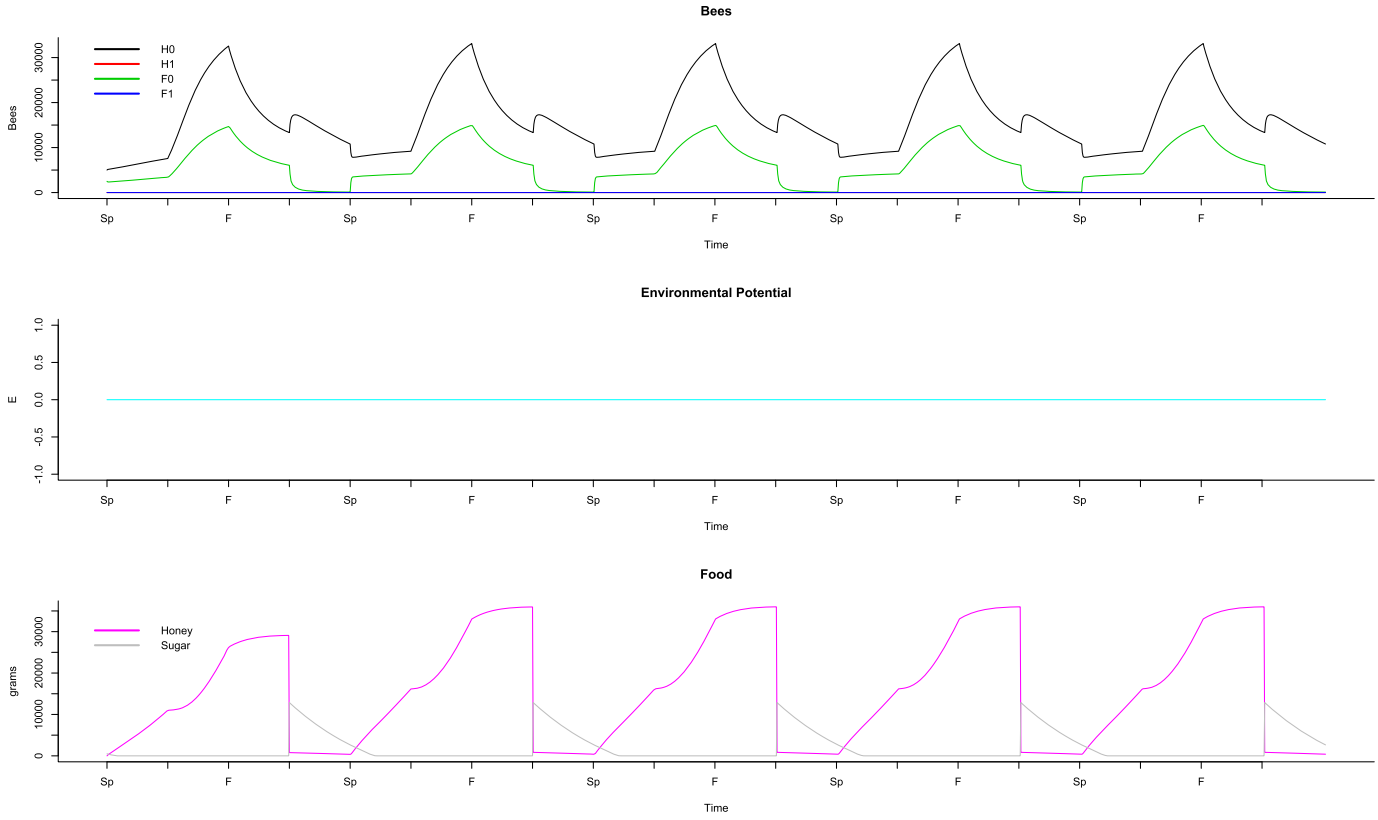


Fig. 4. Disease-free model scenario simulation. The top frame is the dynamics of honey bee population, the middle frame is the environmental potential of disease, and the bottom frame is the dynamics of honey and supplementary sugar. Since the system reaches almost periodic dynamics after the second year, only the first five years of the simulation are depicted in this figure. For this simulation the default parameters in Table 1 have been used.

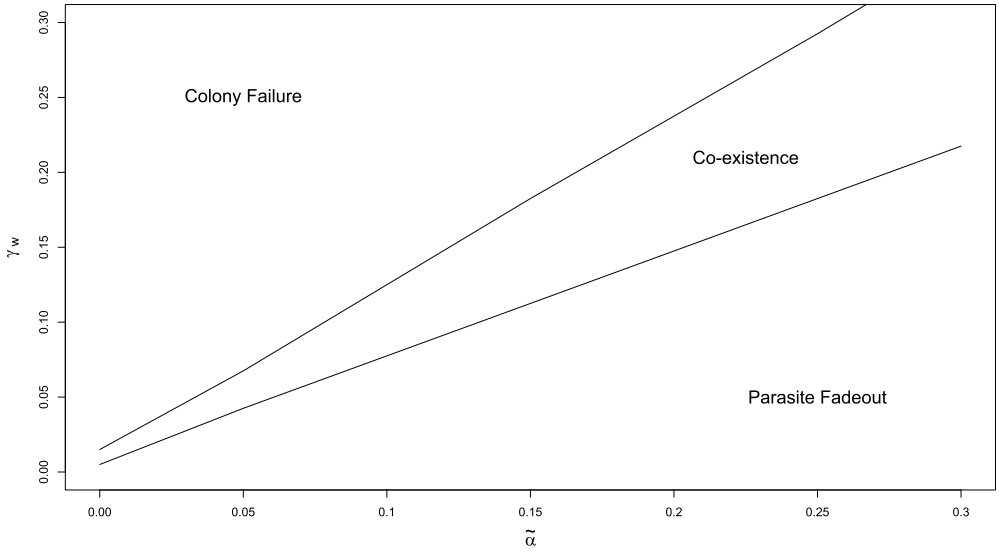


Fig. 5. Curves differentiate between different possible outcomes of the model (disease removal, co-existence, or colony failure) under various combinations of $\tilde{\alpha}$ and γ_w.

Table 2. Cumulative annual percent honey yield loss due to disease.

Year	$\tilde{\alpha} = 0.185$ $\gamma_w = 0.13$ (disease fadeout)	$\tilde{\alpha} = 0.14$ $\gamma_w = 0.12$ (co-existence)	$\tilde{\alpha} = 0.13$ $\gamma_w = 0.13$ (co-existence)	$\tilde{\alpha} = 0.12$ $\gamma_w = 0.14$ (co-existence)	$\tilde{\alpha} = 0.13$ $\gamma_w = 0.166$ (colony failure)
1	0	0	0	0	0
2	1.76	2.85	4.59	7.46	11.17
3	1.49	4.36	12.82	26.22	40.48
4	1.18	5.57	20.57	38.5	53.09
5	0.96	6.63	25.57	45.14	62.7
6	0.8	7.55	28.85	49.52	69.1
7	0.68	8.34	31.16	52.62	73.64
8	0.6	9.00	32.88	54.93	77.02
9	0.53	9.56	34.21	56.71	79.63
10	0.48	10.02	35.37	58.13	81.71

to the hive in the fall, the colony is not strong enough to rebound in the following spring. At amounts of 9650 grams or above, the colony will rebound. If the presence of disease in the colony is weak and would have otherwise resulted in parasite fadeout in the case of food abundance (i.e., using parameters $\tilde{\alpha} = 0.13$, and $\gamma_w = 0.185$), the colony fails when supplementary feeding consists of 9650 grams of sugar syrup. Due to the increased nutritional demand of infected bees, the minimum amount of sugar required for colony survival therefore increases when *N. ceranae* is present.

If a sufficient amount of supplementary sugar is added to compensate for the honey removed in fall, e.g. for the default value of $\mu = 13000g$ (well in excess of the critical value) that is used in many of the simulations that we report here, the starvation terms in the model have only a minor effect on population size and bee loss; they have virtually no effect on the recruitment/reversion between hive and foraging duties (data not shown).

3.3. Increasing supplementary sugar will increase honey yield but probably not at levels that are economically meaningful

In our simulation set-up we assume that honey is harvested at the end of fall. In order to ensure enough nutrition for the colony to survive the winter, supplementary sugar is provided by the beekeeper. Since infection with *N. ceranae* leads to increased food requirements, we investigate the role that the supplementary sugar has on colony strength and honey yield.

We run simulations with the amount of supplementary sugar, μ, between 9 and 14 kilograms, increased in increments of 50 grams. We assume that supplementary sugar is added all at once via a top feeder frame with dimensions the same as a typical shallow super, $19\frac{7}{8}$ x $16\frac{1}{4}$ x $5\frac{11}{16}$ inches, with a small access box measuring $7\frac{1}{8}$ x $4\frac{11}{16}$ x $3\frac{9}{16}$ inches. With one inch thick material, this feeder can hold 975.02 in^3, or 4.22 gallons of syrup. 14 kilograms of sugar is enough to make about 4.325 gallons of 2:1 syrup. This is more than can be held by a standard sized top feeder frame. Occasionally, beekeepers will add additional syrup over the winter if food supplies are limited, although this can be extremely risky as exposing bees to winter cold can have deadly consequences. For the sake of simplicity we assume that supplementary sugar is added only once on the last day of fall.

In Fig. 7 we plot the predicted 10 year honey yield as a function of the amount of sugar added. This includes 6 scenarios: a colony without disease; a colony in the parameter regime in which the disease dies out; three scenarios in which the disease establishes itself in a colony with reduced numbers; and a colony that fails.

We first observe that there exists some threshold value for supplementary sugar addition that is required for colonies to survive. This reflects that after harvesting, not enough food remains available to the colony to survive the winter on their own. In our simulations, we observe this threshold to occur between 9,600 and 9,650 grams of sugar for healthy colonies and between 9,650 and 9,700 grams for colonies infected with *N. ceranae*. These numbers certainly are a result of the specific model parameters, including duration of seasons, that we assumed and cannot be understood as a practical recommendation for beekeepers. They nevertheless suggest that the differences in amount of supplementary sugar required for survival by healthy and infested colonies may be too small to recommend differential strategies.

Once the critical threshold of added sugar is surpassed, the 10 year honey yield increases instantaneously since the colony is able to survive the entire simulation period. As the strength of disease increases, the effect that sugar supplements above the critical threshold have on the 10 year honey yield is reduced. In Fig. 7, this is depicted by the plateau in honey yield with increasing amounts of supplementary sugar. For the stronger colonies (disease free, or parasite fade out), this plateau in 10 year honey yield is observed at approximately 10,500 grams of added sugar, whereas colonies in which the disease establishes itself observes a plateau between 9,800-10,200 grams of added sugar.

For larger amounts of sugar added, the increase in 10 year honey yield becomes linear. Each additional 50 grams of sugar per year (i.e., 500 grams over the 10 year simulation) increases the 10 year honey yield by only approximately 220 grams. Note that this value is based on the parameters mentioned in Table 1. This rate of change appears independent of the parameters $\tilde{\alpha}$ and γ_w that determine the degree of infestation with the pathogen and colony strength. This is likely too small of an effect to be economically meaningful, however, a definite answer to the question of optimal supplementary feeding would require a more in depth economic analysis that is beyond the scope of this research.

To the best of our knowledge there is no data available that would allow a quantitative comparison of our simulation against field observations. Qualitatively the simulations are plausible considering that the primary role of supplementary sugar feeding is to guarantee the survival of the colony during winter, i.e. that this is an intervention with a binary outcome. If a colony survives safely, its actual numbers at the beginning of spring is of minor importance, as shortfalls can be made up.

4. Treatment of *N. ceranae* with the antimicrobial fumagillin

4.1. Introduction and updated model

There are currently few strategies available for controlling *Nosema ceranae* infection since the cessation of fumagillin production in 2018. As mentioned earlier, we acknowledge that the discontinued production of fumagillin will impact the robustness of the results presented here. Instead, we highlight the importance and significance of treatment and merely use fumagillin as an example as it is the only antimicrobial that has been used for the treatment of *N. ceranae* infection. The existing body of research regarding allows for parameterization of the model without relying solely on assumptions.

Fumagillin is administered orally through the consumption of medicated sugar syrup that is added to the colony, typically in fall, or spring depending on the severity of infection [4, 102, 103]. Appropriate application of the chemical can result in immediate and long-term reduction

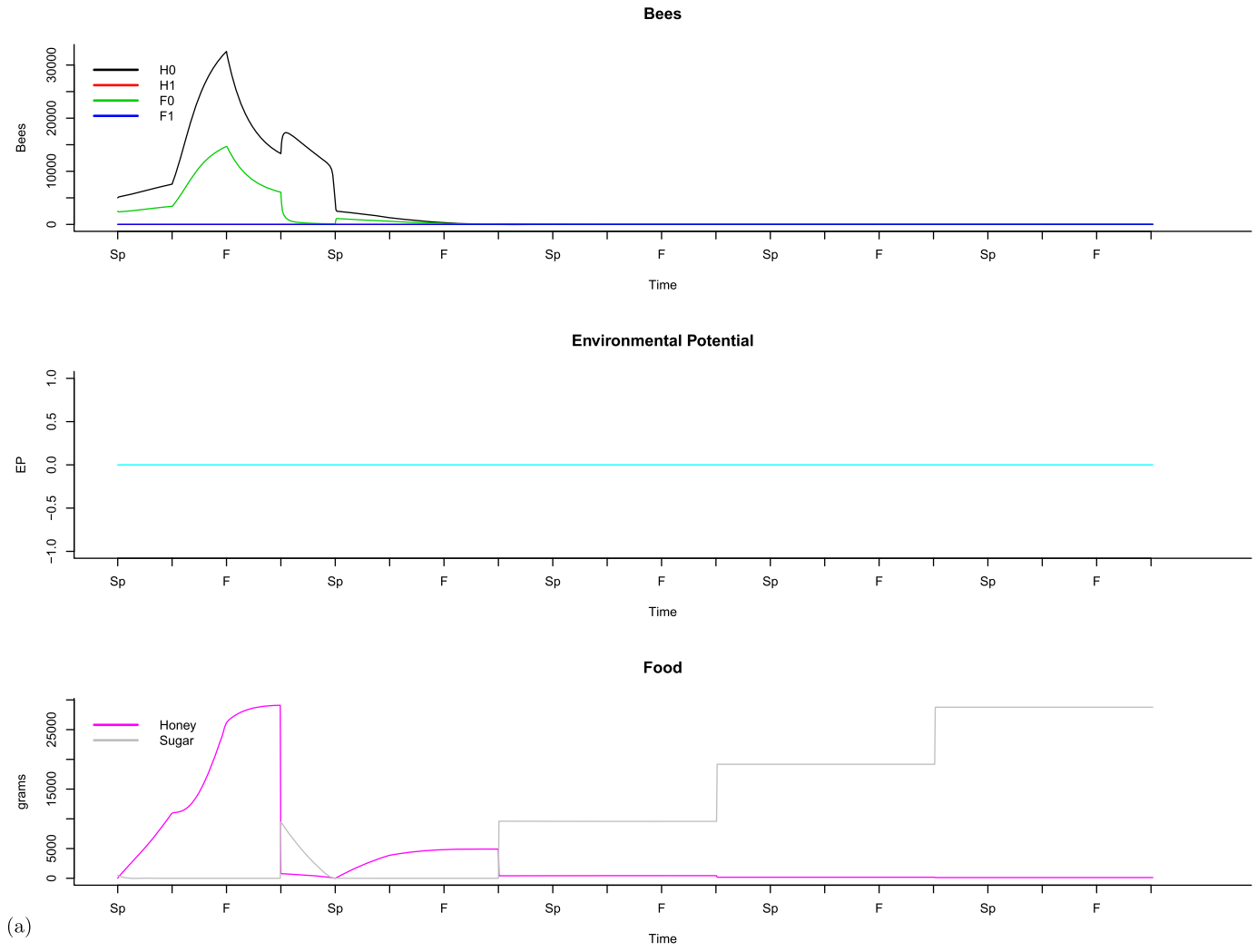


Fig. 6. Effect of supplementary feeding after honey harvesting on model outcome: (a) supplementary sugar feeding of 9600g, absence of disease: colony fails, (b) supplementary sugar feeding of 9650g, absence of disease: colony survives, (c) supplementary sugar feeding of 9650g, mild presence of disease that would fadeout: colony fails.

of infection prevalence [3, 4, 103] and intensity [63, 103], the risk of depopulation can be augmented [3], and colony productivity increased [4, 102]. While the use of fumagillin is effective as a treatment, it is not an effective preventative method as the effects can diminish over time and colonies that have been treated in one year are still susceptible to infection in following years [3, 104]. Because fumagillin is not a vaccination, application when there is a low level of infection may not be worth its cost, especially in autumn when the colony will experience significant natural death anyway [9, 105].

The exact mechanics by which fumagillin affects honey bees infected with *N. ceranae* have not been extensively studied and therefore are poorly understood. One of the only studies to quantify a statistically significant effect of fumagillin on infection intensity observed mean spore count to differ from 8.24 million spores per bee in untreated bees to 2.34 million spores per bee in treated bees [63]. We therefore set the maximum effect of fumagillin to reduce spore intensity, and therefore the deposition of spores: γ, by 75%. This maximum effect is sustained until 33 days after treatment, when spores begin exponential proliferation until they reach a carrying capacity 17 days later.

Below we simulate different treatment schemes for the application of fumagillin to control *N. ceranae* infection in a colony of honey bees. The three schemes include fall treatment, spring treatment, or a combined spring/fall treatment. These can then be compared to the model without treatment, or the model without disease to determine the impact of fumagillin treatment on *N. ceranae* infection. In particular, we are interested in determining not only which treatment scheme is most efficacious, but also the relative differences in honey yield between treatments which might be helpful to determine which may be most economically profitable. We will do this by comparing annual and total honey yield with and without treatments, as well as investigating the effect of treatment on possible model outcomes under various disease parameters $\tilde{\alpha}$ and γ_w.

We make the following model assumptions regarding fumagillin treatment:

1. Once a bee is infected with *N. ceranae*, it cannot be cured with fumagillin. Rather, the antimicrobial reduces the infection intensity and therefore reduces the deposition rate of *N. ceranae* spores into the environment. Fumagillin will reduce infection intensity by a maximum of 75% [63], thereby reducing the number of spores deposited into the environment by the same percentage.
2. The effects of fumagillin are noticeable almost immediately and last for approximately 33 days, at which point the effects wear off and the spores are able to exponentially proliferate in the honey bee. Spore proliferation reaches a plateau after 17 days [25, 28, 29, 30]. In total, the effect of fumagillin treatment lasts for 50 days.
3. Fumagillin is added on harvest day (fall treatment), the first day of spring (spring treatment) or both (combined treatment).

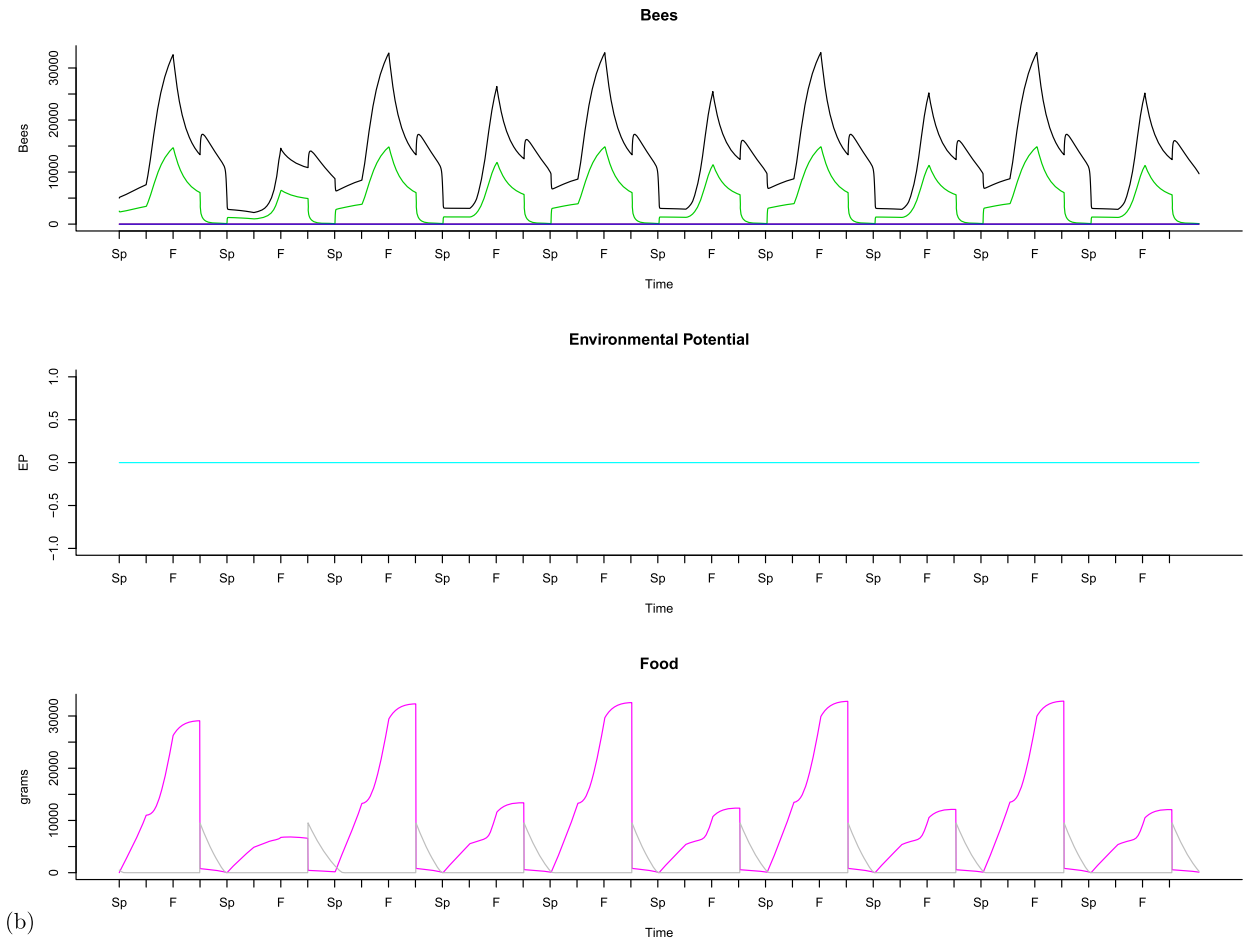


Fig. 6. *(continued)*

This leads to the modified model:

$$\dot{H}_0 = \tilde{\beta}(Z,t) + \sigma\frac{F}{Z}F_0 - R(Z,f,s,t)H_0 - \tilde{\eta}_0(Z,f,s,t)H_0 - \alpha(t)\frac{E}{\lambda+E}H_0 \tag{20}$$

$$\dot{H}_1 = \alpha(t)\frac{E}{\lambda+E}H_0 + \sigma\frac{F}{Z}F_1 - R(Z,f,s,t)H_1 - \tilde{\eta}_1(Z,f,s,t)H_1 \tag{21}$$

$$\dot{F}_0 = R(Z,f,s,t)H_0 - \sigma\frac{F}{Z}F_0 - \tilde{\phi}_0(Z,f,s,t)F_0 \tag{22}$$

$$\dot{F}_1 = R(Z,f,s,t)H_1 - \sigma\frac{F}{Z}F_1 - \tilde{\phi}_1(Z,f,s,t)F_1 \tag{23}$$

$$\dot{E} = \gamma_{fum}(t)\gamma(t)H_1 - \delta(t)E - \tilde{\alpha}(t)\frac{E}{\lambda+E}(H_0+H_1) \tag{24}$$

$$\dot{f} = c_0(t)F_0 + c_1(t)F_1 - \tilde{\theta_0}(f,s,t)(H_0+F_0) - \tilde{\theta_1}(f,s,t)(H_1+F_1) - \theta_B(Z,t) - \xi(f,t)f \tag{25}$$

$$\dot{s} = \mu(t) - \hat{\theta_0}(s,t)(H_0+F_0) - \theta_1(\hat{s},t)(H_1+F_1) \tag{26}$$

New is the addition of the parameter γ_{fum} in (24), which represents the influence of fumagillin treatment on the spore deposition rate. γ_{fum} is a time-dependent, dimensionless parameter, presented in Fig. 8. In accordance with the above assumption we assume that fumagillin reduces the deposition rate by up to 75% over 33 days, after which the effect wanes off. The assumption that the effect of fumagillin is noticeable almost immediately is represented in this function by the exponential decay in γ_{fum} over the first 10 days.

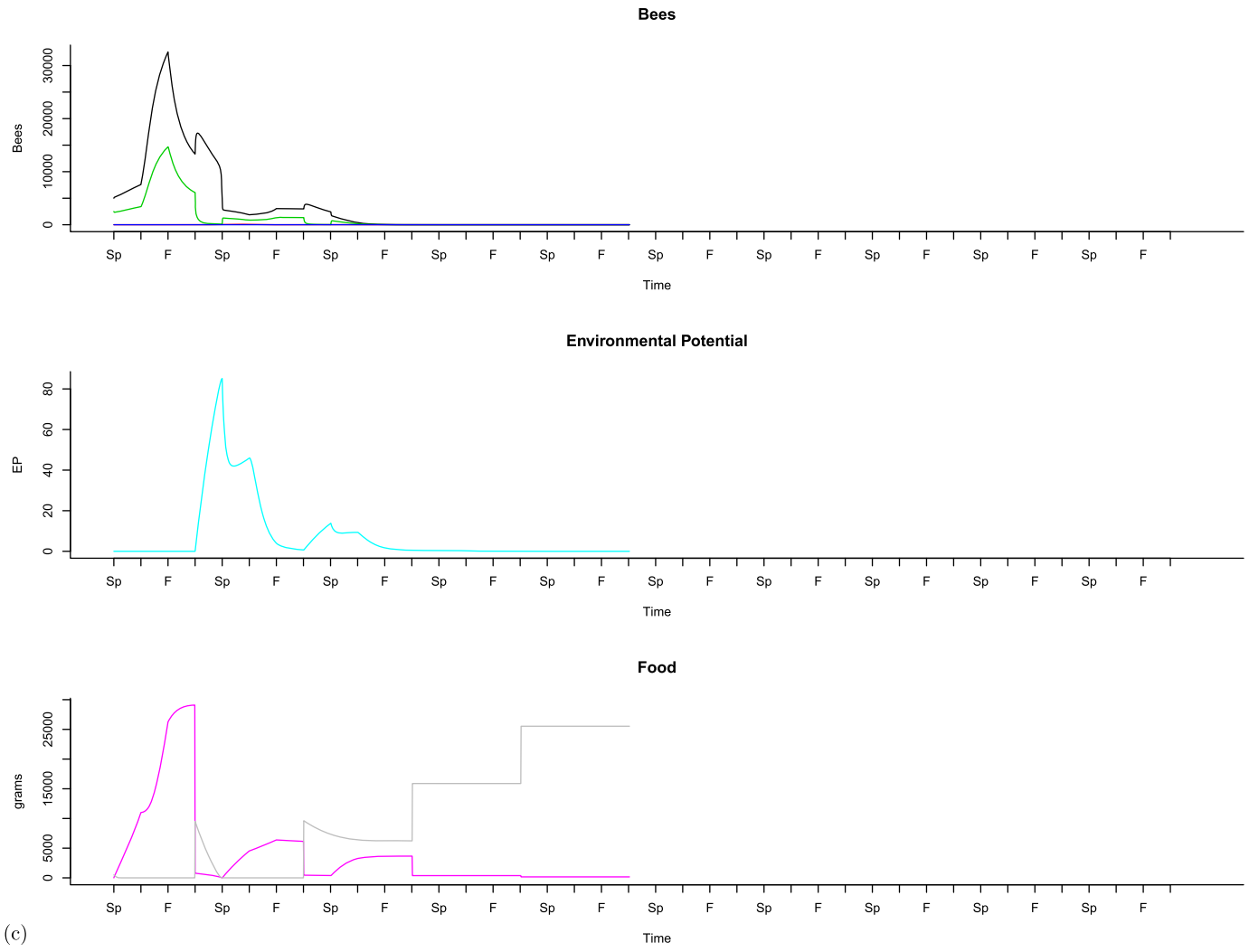
4.2. Fumagillin reduces the number of viable spores in the environment

For our first investigation, we choose the parameters $\tilde{\alpha} = 0.13$ and $\gamma_w = 0.13$, i.e., a scenario in which the disease without treatment will establish itself in the colony. We test the effect of fumagillin treatment on the environmental reservoir of spores in the hive. We expect the environmental potential to be reduced with the application of treatment, but are interested in the differences in environmental potential dynamics between treatments.

As in the case without treatment, we observe a primary peak in environmental potential just prior to spring, and a smaller secondary peak at the beginning of summer. A similar trend is observed in all cases of treatment, however, the size of the primary and secondary peaks are reduced. The proportional differences between primary and secondary peaks of environmental potential differ between treatment schemes, cf. Fig. 9.

For fall treatment, the most noticeable reduction in environmental potential occurs during the primary peak (e.g., 38% reduction in the 6th year). The secondary peak is still reduced compared to the absence of treatment, but reduction (at 15% in the 6th year) is proportionally smaller compared to the first peak. The opposite trend occurs for the spring treatment (69% vs. 78% in the 6th year). Finally, the combination treatment appears to maintain a consistent proportional reduction between the primary and secondary peaks, both being reduced by 96% when compared to the model without treatment. Some of these patterns seem intuitive – if the deposition rates are reduced during winter or spring, one would expect the number of environmental spores to also be reduced at the end of winter or spring. However, the effect of spring treatment on the primary environmental potential peak during the following winter may not seem as intuitive.

Finally, we note that for the spring treatment, the annual dynamics converges slower and does not yet reach periodicity within the first 10 years of simulation.



(c)

Fig. 6. *(continued)*

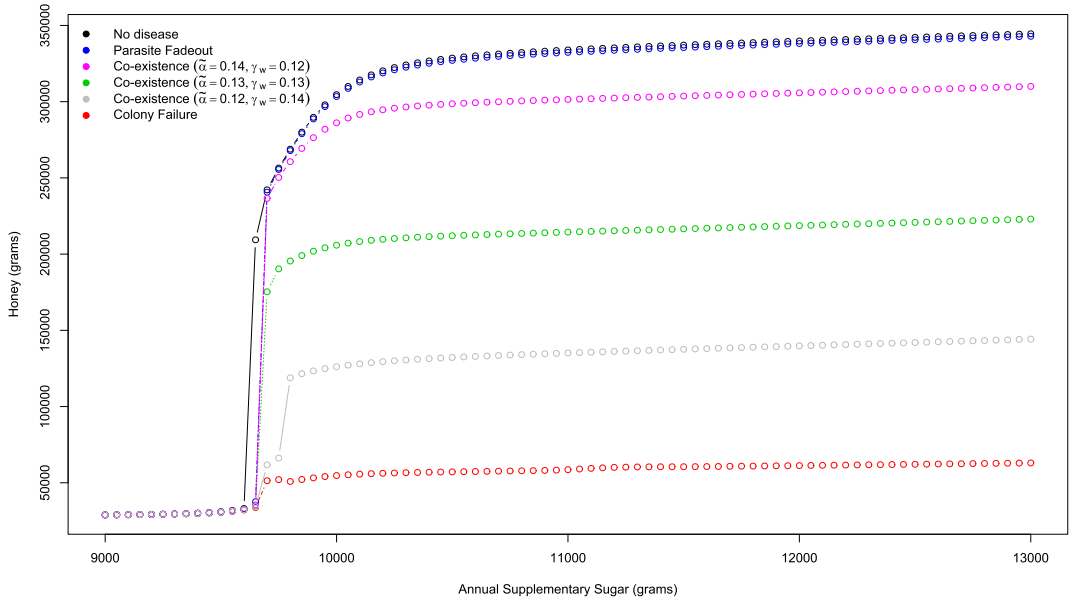


Fig. 7. 10 year honey yield with increasing amounts of supplementary sugar.

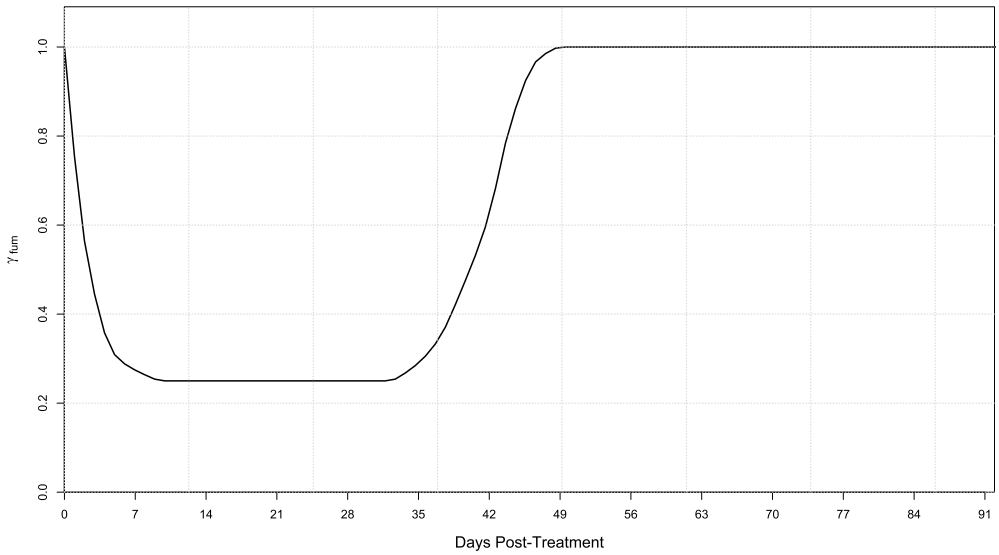


Fig. 8. γ_{fum} function used for the fumagillin treatment at the end of fall.

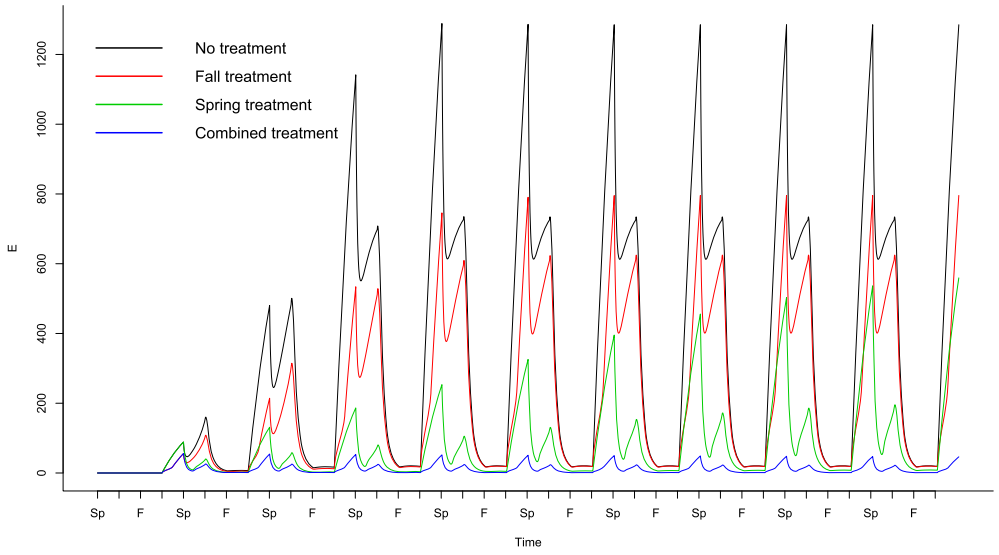


Fig. 9. Environmental potential for each fumagillin treatment scheme in the case of co-existence ($\tilde{\alpha} = 0.13, \gamma_w = 0.13$).

4.3. Fumagillin reduces honey yield loss and risk of failure in colonies infected with N. ceranae

Next, we observe the effects of different fumagillin treatment schemes on the cumulative honey yield over 10 years, first with parameters $\tilde{\alpha} = 0.13$ and $\gamma_w = 0.13$, cf. Fig. 10. As one might expect from the dynamics of the environmental potential presented in Fig. 9, the annual and cumulative honey yields with any kind of treatment are higher than if no treatment is provided. Relative to the model with no treatment, the fall treatment increases the cumulative honey yield after 10 years by 7.95%, the spring treatment by 27.56% and the combined treatment by 33.91%. Relative to the model in the absence of disease, the same treatments correspond to honey yield losses of 27.42%, 7.81% and 1.46%, respectively. While these disease parameters would typically reduce honey yield by 35.37% after 10 years, the effects of *N. ceranae* on honey yield after a combined treatment may even be considered negligible.

We now look to the model evaluated with parameters $\tilde{\alpha} = 0.13$, $\gamma_w = 0.166$ which, without treatment, will cause colony failure (Fig. 11). Similar to the case of co-existence, we observe the largest increases in annual and cumulative honey yield compared to no treatment in order of combined, spring, and fall treatments. We observe the cumulative honey yield curve with no treatment to plateau at year four since the colony has reached a point of failure at this time and no more honey is harvested from the colony beyond this point. However, when any treatment scheme was implemented into the model, the cumulative honey increases over time. This result implies that treatment with fumagillin can save a colony from failure that would have occurred otherwise, which supports the observations made in [3].

We include all values of relative honey percentage loss due to disease under all five disease parameter scenarios in Table 3.

In order to better understand exactly how each treatment scheme influences the possibility of failure, co-existence or disease removal, we run the same simulation detailed in Section 3.2.2. However, we now run the simulation three times, one for each treatment scheme, plus the results with no treatment, cf Fig. 12. With fumagillin treatment, as $\tilde{\alpha}$ increases, the value for γ_w needed to cause colony failure also increases. Additionally, as $\tilde{\alpha}$ increases, there is a higher tolerance for γ_w values that will result in disease removal. In other words, fumagillin treatment can improve survivability of an infected colony as well as

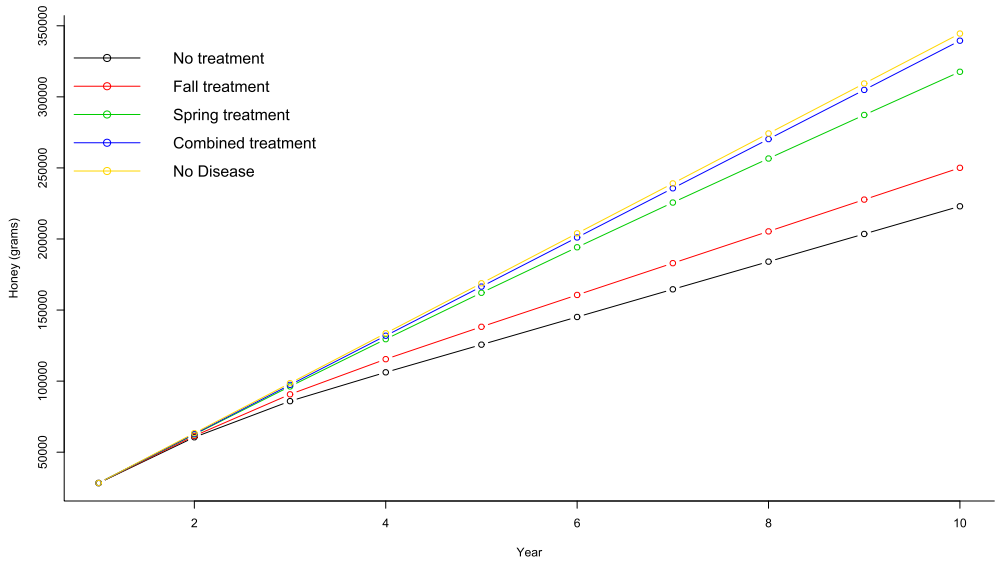


Fig. 10. Cumulative honey yield over 10 years with various fumagillin treatment schemes with disease parameters $\tilde{\alpha} = 0.13$ and $\gamma_w = 0.13$.

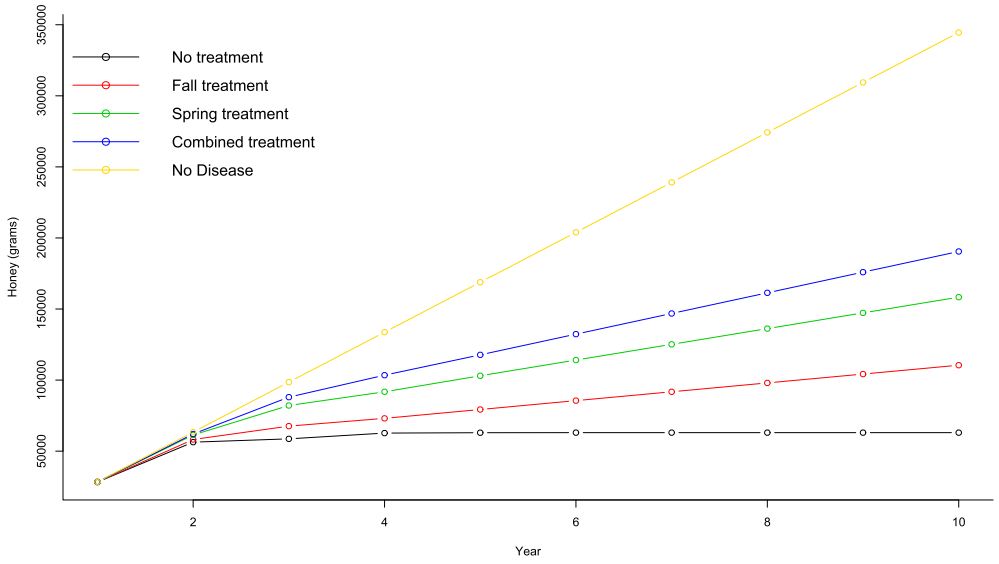


Fig. 11. Cumulative honey yield over 10 years with various fumagillin treatment schemes with disease parameters $\tilde{\alpha} = 0.13$ and $\gamma_w = 0.166$. While the system with no treatment reaches a point at which the cumulative honey yield remains constant, treatment with fumagillin contributes to an increasing cumulative honey yield over time.

its ability to fight off the parasite. In this regard, spring treatment is more efficient than fall treatment, and combined treatment is better than spring treatment alone.

5. Discussion

Honey bee colonies and how they respond to various stressors are often difficult to study under field conditions, both because of challenges to control environmental conditions and to conduct non-disruptive data acquisition. This problem is exacerbated if several stressors interact. Therefore, despite the long cultural history of apiculture and the importance it holds for agriculture, many knowledge gaps on questions of high practical relevance exist. Mathematical modelling is a tool that can often either help in addressing these gaps or to direct more traditional research in an effort toward this goal.

In particular, mathematical modelling of infectious diseases has been established as a powerful tool. For example, at the onset of outbreaks of emerging new diseases, for which only very few data and established facts are known, mathematical modelling can assist in obtaining rough estimates for the expected severity of a developing epidemic, the intensity of an endemic, and to assess the efficacy of intervention and remedial strategies to prevent and mitigate the spread of the disease. Such model predictions always rely on some assumptions that must be introduced, and the result of the model analysis reflects these assumptions. Often these assumptions manifest themesleves in parameter values that are assigned and a sensitivity analysis can show to which extent they affect the quantities of interest. As the disease becomes better understood these assumptions can be corrected, leading to improved model predictions. In the face of quantitative uncertainty of parameters, the predictions made by such models must be understood as phenomenological insight rather than as quantitative estimates. These are thought experiments that allow one to draw conclusions based on the assumptions made. Similarly, owing to the design challenges for field studies there are often no data against which model predictions can be quantitatively validated.

Table 3. Annual percent honey yield loss due to disease with various fumagillin treatment schemes for five combinations of disease parameters. The data are relative to the honey yields of the disease free scenario.

Disease Parameters	Treatment	Year									
		1	2	3	4	5	6	7	8	9	10
	none	0	1.76	1.49	1.18	0.96	0.80	0.68	0.60	0.53	0.48
$\tilde{\alpha} = 0.185$	fall	0	1.14	0.89	0.68	0.54	0.45	0.38	0.33	0.30	0.27
$\gamma_w = 0.13$	spring	0	0.68	0.48	0.36	0.28	0.23	0.20	0.17	0.15	0.14
	combined	0	0.43	0.30	0.22	0.17	0.14	0.12	0.11	0.09	0.09
	none	0	2.85	4.36	5.57	6.63	7.55	8.34	9.00	9.56	10.02
$\tilde{\alpha} = 0.14$	fall	0	1.87	2.39	2.61	2.72	2.78	2.81	2.83	2.83	2.83
$\gamma_w = 0.12$	spring	0	1.00	0.91	0.75	0.62	0.52	0.45	0.39	0.35	0.31
	combined	0	0.64	0.52	0.41	0.33	0.27	0.23	0.20	0.18	0.16
	none	0	4.59	12.82	20.57	25.57	28.85	31.16	32.88	34.21	35.37
$\tilde{\alpha} = 0.13$	fall	0	3.09	7.96	13.65	18.13	21.25	23.47	25.12	26.40	27.42
$\gamma_w = 0.13$	spring	0	1.45	2.32	3.12	3.95	4.80	5.64	6.43	7.16	7.81
	combined	0	0.93	1.20	1.32	1.38	1.42	1.44	1.45	1.46	1.46
	none	0	7.46	26.22	38.50	45.14	49.52	52.62	54.93	56.71	58.13
$\tilde{\alpha} = 0.12$	fall	0	5.22	20.09	31.59	37.91	42.07	45.01	47.20	48.89	50.23
$\gamma_w = 0.14$	spring	0	2.23	8.06	16.68	23.42	27.86	31.00	33.33	35.12	36.56
	combined	0	1.44	4.32	9.30	14.64	18.80	21.82	24.07	25.81	27.19
	none	0	11.17	40.48	53.09	62.70	69.10	73.64	77.02	79.93	81.71
$\tilde{\alpha} = 0.13$	fall	0	8.21	31.40	45.34	53.03	58.06	61.62	64.26	66.31	67.94
$\gamma_w = 0.166$	spring	0	3.20	16.67	31.36	38.97	44.07	47.66	50.33	52.40	54.04
	combined	0	2.10	10.71	22.62	30.25	35.13	38.58	41.15	43.13	44.72

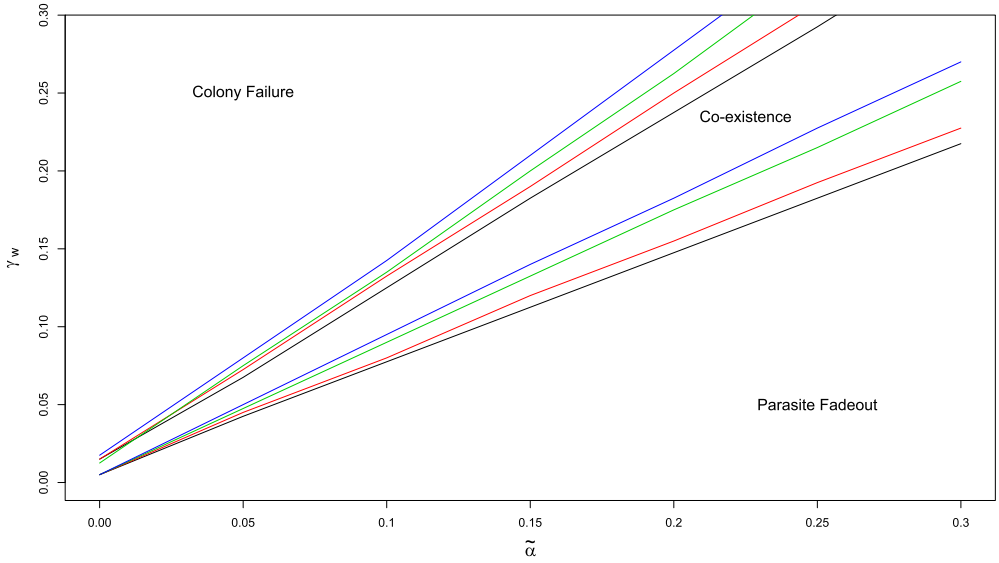


Fig. 12. Possibility of model outcomes under various values of $\tilde{\alpha}$ and γ_w. The black lines are the division lines without treatment, the red, green and blue lines are division lines for fall, spring and combined treatments, respectively.

Using mathematical modelling, we studied the combined effect of *Nosema ceranae* and food storage on honey bee population dynamics. Sugar syrup, which is often added to a hive following honey harvest to ensure survival of the colony, may be fully consumed over the span of winter. The dynamics of food storage at this time are important as shortages may then become a contributing factor to wintering losses that are often observed in colder climates. Colonies already weakened by dwindling food supplies may further be stressed by the presence of *Nosema ceranae*, which usually peaks in prevalence towards the end of winter when spores are ingested during hive cleaning activities.

Our model builds on and combines the two previous models that each account for one of these two stressors. The disease model was adapted from [12] without modification. While [11] accounted for the effects of potential food store short falls, it did not account for honey harvesting and subsequent supplementary feeding. These aspects were newly introduced into the model here. Due to the lack of field data, an important assumption that needed to be introduced was the question of whether bees consume more food during the winter. Whereas calorimetric data for winter exist, such information is much more difficult to gather for summer conditions. Aside from the difficulty of gathering this data from the field, consider also that bees consume food during foraging flights which would reduce the amount of food needed to be consumed from the hive. We made here the assumption that food demand in winter is higher than in summer, as the temperature in the hive's core needs to be maintained at 21^{o}C, well above exterior temperature. See also the review of relevant literature on models of thermoregulation in bee colonies in [106]. For colder climates, such as Canada and the Northern parts of Europe this seems reasonable. The results that we find might be entirely different if this is not correct.

Simulations presented here suggest that the *N. ceranae* model with food storage dynamics and seasonal effects, like the simpler underlying autonomous disease model that was investigated in [12], permits three potential outcomes: (i) Removal of the disease (here referred to as parasite fadeout), (ii) endemic disease in which the *N. ceranae* is firmly established in the population, and (iii) colony failure due to the disease. These outcomes are reflected in the honey yield of the colony. That is,

the more pronounced the parasite establishes itself in the colony, the lower the honey yield. The transition between these regimes in simulations was studied by varying two of the key parameters of the disease – the deposition of spores by infected bees and the uptake rate of spores during cell cleaning.

Our simulations of the disease aspect of the model seem somewhat intuitive. When the rate of removal of spores during hive cleaning is sufficiently high (or the spore deposition rate sufficiently low) the disease is removed faster than it is able to spread, and the disease eventually becomes non-existent. When the uptake rate and deposition rate are approximately similar, this is when the disease can establish itself in the colony. These results possibly help explain inconsistencies between the research studies regarding the effect of *N. ceranae* on honey bee colony populations. While some observe *N. ceranae* to play a critical role in colony failure, others suggest that *N. ceranae* is more of a sub-lethal stressor, that, when combined with other stressors, can cause colony failure. And still, some claim that *N. ceranae* is not an issue. It is entirely possible that the differences between these studies can be explained by variation in geographic, apiary or even colony-specific parameter values for spore deposition and uptake. Genetic differences between bees from different geographical regions, such as those observed in the Danish breed of *Nosema*-tolerant bees, may also affect these parameters and therefore explain differences in prevalence between certain areas around the world.

A more thorough analysis of the disease dynamics could be attempted, using the established techniques from the theory of dynamic systems, next generation matrices, etc. Considering the sheer number of parameters in the model and the fact that they are time dependent periodic, we expect that the limitation of a rigorous analytical approach will be reached rather soon, and that such analysis will have to rely on sophisticated numerical bifurcation tools, and will be rather involved. We believe that for the question at hand, however, the detailed bifurcation structure of the underlying disease model does not need to be known. Of relevance is the endemic regime. In the failure regime questions of food storage dynamics, honey harvesting, etc. become meaningless. On the other hand, in a regime where the disease will fade out, the honey bee colony behaves after short time like a healthy colony, and honey yield and food demand will be unaffected by the disease.

The dynamics and sizes of honey bee populations produced from disease-free model scenario simulations are realistic and are consistent with observations made in the field. Populations peak at approximately 48,000 bees during summer and maintain an approximate 1:2 forager to hive bee ratio before reaching a trough of approximately 11,000 bees during winter. The honey yield from the first year is slightly less than that of subsequent years, which is also observed naturally. In fact, the honey yield that we observe from our model is much higher than what is typically harvested from a colony in its first year, in which it is not uncommon to harvest very little or no honey. This is attributed to the initial conditions that we set for the model, i.e., a transient effect. If we set our initial populations to be half the size as described in Section 2.3, the honey yield in the first year is only approximately 5kg.

To investigate the combined effect of disease and supplementary sugar feeding on honey yield and population dynamics it was necessary to include several new aspects in the model that are not included in either of the two earlier models on which our study is built. One is the removal of honey as a sink for food stores and a second is the addition of sugar as a source for food stores. To be able to account for preferences between food types, it was necessary to include sugar as a separate dependent variable. Thirdly, it was necessary to include starvation terms in the population model. In scenarios of food abundance, for example if no honey is harvested or supplementary sugar is administered in excess of the critical required amount, these starvation effects have only negligible effect on the population size, as one expects. However, when food becomes limited – which in our study can be the case at the end of winter if not enough sugar was added to compensate for the removed honey in fall – these starvation terms become a key component of the model and can drive the colony toward failure. Since *Nosema* infected bees have a higher nutritional/energetic requirement, the level of disease infestation plays a role here as well. This, however, is not very pronounced at mild to moderate infection levels but becomes more relevant if the infestation is in the failure regime. The fourth important aspect of food dynamics comes from considering that food supply might affect recruitment to foraging duties and reversion to hive duties, as modelled in [11].

Both stressors, *N. ceranae* and potential food shortfall during winter, can individually and concurrently lead to loss in honey yield: The four most sensitive parameters for this quantity of interest include the two parameters that describe disease transmission, namely the spore deposition rate and the spore ingestion rate, as well as the in-hive food uptake rate by the colony's brood, and the rate at which forager bees die. The first two parameters show their main effect in winter and spring during hive cleaning in preparation for the new brood, where disease transmission is most active. The other two parameters are primarily active during spring, summer, and fall when brood are being tended to and foraging flights occur.

Another key parameter in our model is the amount of supplementary sugar that is provided to the colony before winter after honey harvesting in order to compensate for the honey removed. The effect of this parameter is almost binary – there exists a critical threshold of supplementary sugar below which the colony will not survive. If this threshold is exceeded, honey yield increases first with the sugar amount and then levels off. In colonies with a high *N. ceranae* infestation this transition is rapid, in an on/off fashion, but honey yield can be low. In healthy colonies and colonies with mild infestation, the dependency of honey yield on sugar supply is more differentiated. If the critical threshold is exceeded, then honey yield increases smoothly with amount of sugar feeding over a short interval, before it levels off. The actual sugar supply threshold changes mildly with the level of *N. ceranae* infestation, but in all parameter cases tested it remained within a narrow range. Our simulations suggest that supplementary sugar feeding, beyond ensuring that the colony survives the winter, cannot compensate for honey loss due to disease.

We further investigated if treatment of an infected colony can restore the loss of honey yield due to disease. Our simulations suggest that in cases of colonies with moderate *N. ceranae* infestation this indeed might be possible, as remedial strategies shift the transition points between the endemic and disease-free regimes. In cases of severe *N. ceranae* infestation such treatment cannot prevent the losses but shows substantial improvement vis-a-vis untreated colonies under the same conditions. The mitigation strategy that we implemented mimics the effect of fumagillin, namely a reduction in spore deposition rates for a limited period of a few weeks after treatment. Our simulations suggest that applying this treatment at the beginning of spring might, with view on honey yield, be more efficacious than an application at the end of fall. This was not *a priori* predictable because the spore deposition rate is larger during winter than during spring. On the other hand, the spore ingestion rate by worker bees is greater in spring. If applied at both times in mildly to moderately infested colonies much of the honey lost due to the disease without treatment can be recuperated. In colonies that are heavily affected some recuperation is possible but far from the levels of a healthy colony. While more frequent treatments may remediate the effects of honey loss due to disease, determining optimal profitable treatment strategies would require an economic analysis that goes beyond the scope of our population and disease dynamics modelling study.

Colony collapse disorder (CCD) is the failure or severe weakening of a colony that is characterized by the following: the ratio of brood to adult bees is abnormally high; dead workers are not found in or around the hive; and the invasion of kleptoparasites, who steal the honey and pollen stores that remain in the hive, is delayed [1]. Based on our model, we cannot comment on the state of relative brood population or parasites other than *N. ceranae*. However, unlike most existing mod-

els of honey bee diseases, such as [12, 73, 74, 75, 76, 77, 79, 80], we are able to keep track of the amount of stored food in a hive at the time of colony failure. With the model evaluated with parameters $\tilde{\alpha} = 0.13$ and $\gamma_w = 0.166$, the colony reaches a point of failure on day 1584, or the $33rd$ day of summer in the fourth year. On this day, there is approximately 436 grams of honey and 12,900 grams of supplementary sugar remaining in the hive. The delay of kleptoparasite invasion in CCD means that post-collapse, there should be remaining food stores, which we do observe from simulations of our model in which colony failure occurs. This does not imply that *N. ceranae* is the cause of CCD, but it does suggest a possibility that it may be a contributing factor to colonies suffering from CCD. Furthermore, the fact that colonies can reach a point of failure with remaining food stores suggests that *N. ceranae*-infected colonies likely do not fail due to starvation. In fact, in the 120 days prior to failure, the colony persists with very low overall populations in our simulations. It is possible that *N. ceranae*-induced colony failure is due to an Allee-like effect that occurs in honey bee populations, as a sufficiently large number of worker bees is required to maintain the brood. The colony reaches a critically low population from which it is unable to recover from and fails shortly thereafter. We suggest that in addition to food stores, a model including brood dynamics may better determine whether or not *N. ceranae* infection can produce characteristics similar to those observed in colonies with CCD.

6. Conclusion

Nosema ceranae is a common gastrointestinal parasite of the western honey bee that leads to increased nutritional and energetic requirements of infected individuals. It is not well understood how it affects a colony's honey production and its requirements for supplementary sugar during winter seasons, and how both stressors – disease and potential food limitations – amplify each other. To explore these questions we combined two previous mathematical models, one of *N.ceranae* disease dynamics and one of population and food dynamics. This combined model was then extended to account for honey removal in autumn and subsequent supplementary sugar feeding to provide to the colony sustenance during the winter. This results in a model of seven coupled non-linear differential equations with time periodic coefficients. The complexity of this model prevents a rigorous theoretical analysis of the model dynamics. Therefore, we investigated its qualitative behaviour in numerical simulation experiments, with a focus on honey yield and supplementary sugar requirements of a colony in which *N. ceranae* is endemic. For some parameters reliable values can be found in the literature, whereas for others no such data is readily available. This introduces considerable uncertainty in the quantitative prediction of the model. Therefore, the focus of our modelling study is the qualitative phenomenological exploration of the combined effect of disease and possible food shortfall.

There is a critical amount of sugar that needs to be provided after honey removal, below which the colony will not be able to survive the winter. The exact value of the critical amount depends on the level of disease infestation in the hive but lies in a range that might be too narrow to be of practical relevance for the optimisation of hive management strategies. If sugar is supplemented in excess of this critical value, so that a functioning colony survives the winter, the honey yield increases due to the increase in colony strength at the beginning of spring, but eventually appears to level off. Under the model assumptions that we introduced, we find that this behaviour is qualitatively the same but quantitatively different for healthy colonies and endemic colonies that are mildly or moderately affected by the disease. The more prevalent the disease, the lower the honey production. Presumably this is a direct consequence of the increased nutritional requirements and less productive foraging flights of infected bees. Indeed, a sensitivity analysis of the model has shown that among the four parameters that are most sensitive for honey yield are two that are connected to disease transmission, and two that are connected to food dynamics, which suggests that both stressors play an important role for honey loss. Our simulations suggest that for colonies in the moderate endemic regime, disease mitigation strategies that induce temporary reduction of spore deposition in the hive might be able to prevent a substantial fraction of honey loss compared to a non-treated colony with the same infestation levels.

Declarations

Author contribution statement

J. R. Comper: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

H. Eberl: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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