Interactions between immunotoxicants and parasite stress: implications for host health

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Abstract

Many organisms face a wide variety of biotic and abiotic stressors which reduce individual survival, interacting synergistically to further reduce fitness. Here we studied the effects of two such synergistically interacting stressors; immunotoxicant exposure and parasite infection. We model the dynamics of a within-host infection and the associated immune response of an individual. We consider both the indirect sub-lethal effects on immunosuppression and the direct effects on health and mortality of individuals exposed to toxicants. We demonstrate that sub-lethal exposure to toxicants can promote infection through the suppression of the immune system. This happens through the depletion of the immune response which causes rapid proliferation in parasite

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load. In addition, high toxicant exposure can alter cellular regulation and cause the breakdown of normal healthy tissue, from which we infer higher mortality risk of the host. We classify this breakdown into three phases of increasing toxicant stress, and demonstrate the range of conditions under which toxicant exposure causes failure at the within-host level. These phases are determined by the relationship between the immunity status, overall cellular health and the level of toxicant exposure. We discuss the implications of our model in the context of individual honey bee health. Our model provides an assessment of how pesticide stress and infection interact to cause the synergistic breakdown of the within-host dynamics of individual honey bees.

Keywords: stress, immunity, infection, synergistic, within-host dynamics, honey bees

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1. Introduction

During their lifetime, organisms are exposed to a wide range of chemical, physical and biological stressors. Exposure to environmental (e.g. anthropogenic, climatic) and natural stress (e.g. pathogens, parasites and predation) reduces individual fitness [1]. Recently, there has been increasing interest in multiple stress approaches, examining the potential for stressors to interact synergistically, defined as the combined effects of stress having a greater impact than expected [2]. Understanding the mechanisms behind these synergistic interactions is important for quantifying the true impacts of individual anthropogenic stress on organisms [3].

Pesticides are an important class of anthropogenic toxicant stress, with the use of pesticides continuing to increase globally [4, 5, 6]. Pesticides are seen as crucially important to crop productivity, preserving around one-fifth of total crop yield contributing to food security [7]. Concerns about the detrimental impacts of these pesticides [8, 9] have in the past forced policy makers to restrict the application of some insecticides [10]. Non-target insects frequently encounter these insecticides [5], with concentrations able to build up throughout food sources and within various life-stages of the organism [11, 12, 13, 14, 15, 16, 17].

Toxicants such as pesticides can cause lethality [18, 19, 20], but more often have other sub-lethal effects such as impairments on foraging [21, 22, 23, 24], feeding [25], learning [26, 27], memory [28, 27] and fecundity [29, 30, 31]. Exposure during early life can have both lethal and sub-lethal effects later

appearing during adulthood [32, 33]. These environmental contaminants can interact synergistically in combination with other natural stressors. For example, combinations of toxicant exposure with parasite infections can increase individual mortality [34, 35], increase the initial pathogen load [36, 37] and increase virulence [38]. Synergistic toxicant-pathogen interactions have been observed in many types of organisms such as insects, snails, water fleas, frogs, salamanders, fish and mussels (see review by Holmstrup et al., 2010). In addition to toxicants causing direct lethality, they can also cause indirect damage to individual immune defence. Individual organisms defend themselves against various infections via a suite of immune responses, and these can be damaged or inhibited through toxicant exposure [39]. For example, pesticides have been shown to reduce the total hemocyte abundance in insects [40, 41], the nodulation initiation [40, 42], the encapsulation response [43, 41] and antiviral defences [44].

Of particular recent concern are the widespread losses to global wild and managed honey bee populations [6, 45, 46]. The Western honey bee (*Apis mellifera* L.) is widely recognised as the most important commercial insect pollinator [47, 48, 49, 50], contributing to global food security and biodiversity [51, 52]. While a single cause for these widespread colony losses has yet to be identified, there is agreement that it may have its origins within multiple stressors interacting with each other [53, 54, 55, 56]. Possible candidates include neonicotinoid pesticides [12, 13, 57, 27], mites [58, 59], viruses [60, 61, 62] and microsporidia infections [63, 64].

In this study, we examine the general mechanism by which immunotoxicants interact with infection to reduce host health. This observed synergy between multiple stressors is currently poorly understood from an immunological perspective [65]. We focus our study on the general ecotoxicological applications of the model, in the case of any immunotoxicant interacting with any parasite infection. We do this by formulating a system of nonlinear ordinary differential equations (ODEs) to investigate the consequences of immunosuppression by a toxicant and the effect this has on within-host infection. We first consider a toxicant-free environment to examine the conditions under which the infection can spread. We then consider the interaction between the infection and both lethal and sub-lethal exposure to toxicants and examine the outcome on within-host dynamics. We also consider the case of aggressive direct lethality of toxicants on the production of new tissue cells.

2. The Model

The immune response of any individual relies upon the interdependent defence of physical, humoral and cellular responses, denoted in our model by immune function Z. Nowak and May [66] proposed a general model to describe the interaction between a cellular immune response and a replicating virus, in the setting of self-regulating cytotoxic T lymphocytes (CTLs) targeting infected cells. The model they present is simple but captures the fundamental biological processes governing the immune response to foreign antigens, and following this framework we denote within-host cell density as

X. We denote the total parasite/pathogen density as Y. The total number of cells within the model represents a general susceptible subset of animal tissue cells. As a motivating example, our model can be thought of describing the midgut epithelial cells of the honey bee X under a *Nosema ceranae* infection Y [67] with associated immune response Z, although we also propose that our model can be thought of describing any interaction between any immunotoxicant and associated parasite or pathogen in a general host.

Toxicants can be lethally toxic to individuals at high enough exposure [18, 19, 20]. In addition various functions associated with the immune response are damaged by toxicants [39, 40, 41, 42, 43, 68, 69, 70, 44]. We model both the direct lethality (denoted by parameter r) and indirect sub-lethal immunotoxicity (denoted by parameter h) effects of toxicant exposure Q. For simplicity, we assume fast dynamics of virus replication compared to the replication of other immune or within-host cells resulting in the formulation of the model (Figure 1) as a 3-compartmental set of nonlinear ODEs;

$$\frac{dX}{dt} = \lambda - \beta YX - dX - rQ \tag{1a}$$

$$\frac{dY}{dt} = \beta YX - aY - pYZ \tag{1b}$$

$$\frac{dZ}{dt} = c - bZ - hQ \tag{1c}$$

with $c - hQ > 0$ and $\lambda - rQ > 0$. When $Z = 0$ (the immune response is depleted), we remove equation (1c) from system (1) and the system becomes

the two dimensional system of equations (1a) and (1b) without the immune response term $-pYZ$;

$$\frac{dX}{dt} = \lambda - \beta YX - dX - rQ \tag{1a}$$

$$\frac{dY}{dt} = \beta YX - aY \tag{1b}$$

We assume that within-host cells are produced at rate λ, and die at per-capita rate d. Parasites are created at rate β via a linear mass action, and are removed at per-capita rate a. The immune response Z is activated upon encountering parasites Y and the removal of parasites occurs at rate p. Although in reality, functions involved in immunity are not activated on the instance of meeting the parasite, but there is a complicated intermediary chain between processes which eventually result in the removal of parasites [71]. For simplicity, we assume that this complicated process can be summarised by our function pYZ. We assume that the immune dynamics Z are decoupled from those of within-host and parasite density. This represents the simplest possible assumption and various extensions to this assumption are possible. Immunity is therefore produced at rate c, and is removed at per-capita rate b.

Within our model we infer the mortality risk of the host through the status of the within-host cells X, so that individual mortality risk is high when the number of cells X is small. This condition enables us to think about the mortality risk of an individual analogous to a highly infected within-host

tissue (e.g. parasite infection within the gut of a honey bee).

Our system of equations (1) were analysed using standard stability methods from dynamical systems theory and solved numerically with Wolfram Mathematica version number *10.0.2.0*, using parameters taken from Table 2. We performed a full parameter dependence analysis which demonstrated the same two universal behaviours of the model which enabled us to choose arbitrary parameter sets.

3. Results

In the following section we consider the baseline case of parasite infection in a toxicant-free environment before analysing our within-host system under the addition of a toxicant. We then consider the absence of direct lethal effects of toxicants before presenting the unique case of an aggressive toxicant.

3.1. Toxicant-free model

Initially we examine system (1) under the condition of the absence of toxicant exposure (denoted by subscript A). Two possible outcomes are possible. First the infection is removed entirely by the immune system, in which case the total within-host cells and total immunity each reach a constant level at the disease free equilibrium (DFE):

$$(X_A^{DFE}, Y_A^{DFE}, Z_A^{DFE}) = \left(\frac{\lambda}{d}, \quad 0, \quad \frac{c}{b}\right) \tag{2a}$$

where $\frac{\lambda}{d}$ and $\frac{c}{b}$ represent the ratio of total production to total removal of both within-host cells and immunity in the absence of toxicant respectively. Secondly the model predicts that an individual can become infected with parasites ($Y > 0$) under the following endemic equilibrium (EE):

$$(X_A^{EE}, Y_A^{EE}, Z_A^{EE}) = \left(\frac{ab+cp}{\beta b}, \quad -\frac{d}{\beta} + \frac{b\lambda}{ab+cp}, \quad \frac{c}{b}\right) \tag{2b}$$

This shows that it is possible for an individual honey bee to sustain a partial parasite infection without the addition of any toxicant in our model. The expression $\frac{ab+cp}{\beta b} = \frac{a}{\beta} + \frac{cp}{\beta b}$ represents the reduction in within-host cells.

3.2. Toxicant-Parasite model

Next we consider system (1) under the condition of an infection and toxicant exposure (denoted by subscript B). In this case the model predicts two possible outcomes. First, the parasite infection is removed either by immune suppression or by the direct effects of the toxicant on the production of within-host cells represented by the DFE:

$$(X_B^{DFE}, Y_B^{DFE}, Z_B^{DFE}) = \left(\frac{\lambda - rQ}{d}, 0, \frac{c - hQ}{b}\right) \tag{2c}$$

so that the addition of any toxicant reduces the total within-host cells by $\frac{rQ}{d}$ and reduces the immune function by $\frac{hQ}{b}$. Secondly the model predicts an

infected individual under toxicant exposure represented by the EE:

$$(X_B^{EE}, Y_B^{EE}, Z_B^{EE}) = \left(\frac{ab + cp - hpQ}{\beta b}, \frac{-abd - cdp + dhpQ - bQr\beta + \beta b\lambda}{\beta ab + cp\beta - hpQ\beta}, \frac{c - hQ}{b}\right) \tag{2d}$$

In this case, the parasite density grows rapidly as a result of the toxicant suppressing the immune system. The introduction of the toxicant reduces both within-host cells and immunity in both an infection-free and infected individual, but an initial parasite infection is required for an infection to grow.

The effect of toxicant exposure on the net change of within-host cells, parasite density and immunity within the individual is summarised in Table 1.

Next we assume that the indirect (sub-lethal) effects of toxicant exposure on immunosuppression are more prominent than the direct (lethal) depletion of within-host cells. With an initial infection $Y > 0$ we define this as occurring when the immune status of an individual is destroyed before the infection is removed or when

$$Z = 0 \quad \text{before} \quad Y = 0 \tag{3}$$

We summarise the behaviour of the model under this condition (Figure 2) into 3 distinct phases which describe the mechanism underlying the interaction between toxicant exposure and infection at the within-host level of the

organism, and the parameter dependence of infection and immunity at equilibrium. Note that the total number of cells within an individual organism is not constant. This is because both parasite and within-host cells are removed by either the toxicant exposure or infection and new cells are produced.

Phase I $\quad 0 \leq Q < \frac{c}{h} = Q_0^*$

The model predicts that the initial state of an immune response is able to counter any infection. However, as the toxicant load is increased, the immune system is gradually depleted. Through a weakened immune suppression, this enables the parasite density to increase.

Phase II $\quad Q_0^* = \frac{c}{h} \leq Q < \frac{\beta\lambda - ad}{r\beta} = Q_1^*$

The second phase begins at the point of maximum infection and where the immune system has been completely inhibited. The increase in toxicant stress gradually depletes the parasite density while the within-host cells remain constant.

Phase III $\quad Q_1^* = \frac{\beta\lambda - ad}{r\beta} \leq Q$

In phase three, the immune system has been destroyed and the parasite infection is no longer present leaving only a small fraction of within-host cells. Finally, the lethality of the toxicant causes the mortality of the individual honey bee and production of new cells ceases.

Thus we have calculated the conditions under which the within-host dynamics change according to the level of toxicant exposure. By understanding

the relationship between the parameters in the model and toxicant stress, we can make some biological interpretations. We predict that the ratio of the production of immunity to the amount of immunotoxicity ($Q_0^* = \frac{c}{h}$) determines the point at which the infection load is at a maximum. The expression $\frac{c}{h}$ can be thought of as an indicator of immune status, and the point at which the toxicant stress becomes equal ($Q = Q_0^*$) represents the complete inhibition of the immune system. The expression $Q_1^* = \frac{\beta\lambda - ad}{r\beta} = \frac{\lambda}{r} - \frac{ad}{r\beta}$ represents the point at which the ratio of cell production to lethal toxicant mortality (indicator of within-host cell status) compares to the ratio of the loss of cells to the toxicant cell depletion multiplied by the transmission of the infection. Therefore this condition represents the status of within-host dynamics and can be thought of as an indicator of health. When $Q = Q_1^*$, the infection has been removed but the overall health status is very low, from which we infer a higher mortality risk of the host. Therefore we have conditions describing how toxicant exposure relates to that of the immune status Q_0^* and overall health Q_1^* of the organism.

Our model predicts that a small amount of toxicant can cause the outbreak of an otherwise controlled infection. A healthy immune response can suppress the parasite infection to a very low level (Figure 3.a), but a small amount of toxicant can cause the status of both infection-free and infected individuals to decline rapidly (Figure 3.b).

3.3. Absence of toxicant lethality

In this case, we consider the absence of a direct lethal toxicant effect, therefore assuming that toxicant exposure only impairs the immune system and does not cause direct mortality. This changes the mechanism by which organisms become infected under increasing toxicant exposure. As before the immune system is inhibited leaving the organism vulnerable to attack by parasites. However after reaching a maximum infected threshold, the health status of the individual remains constant regardless of the amount of toxicant exposure (Figure 4.a). The individual remains highly infected (Figure 4.b) and an increasing exposure to the toxicant no longer causes further damage to organism health status.

3.4. Aggressive direct mortality

It is worth noting that condition (3) is necessary to explore the interaction between toxicant immunosuppression and the immune system. If this were not the case, for example if the parameter r becomes large we would see a situation where the toxicant acts too aggressively upon the host and causes the parasite infection to be killed off (similar to phase II under the original assumption) and following this the within-host cells are destroyed. The immune system remains intact as the direct effect of the toxicant on production of within-host cells is greater than the immune effect. We again see three distinct phases as we increase the toxicant from low levels to high (Figure 5a). However now the toxicant exposure is more prominent and reduces both

parasite and within-host cells, stopping the infection from spreading quickly (Figure 5b). In this situation we also see a somewhat contradictory phase 3 in which the host has neither parasite or within-host cells but a small amount of immunity. This result demonstrates the necessity of our original condition.

4. Discussion

We have shown that interactions between general anthropogenic stress in the form of an immunotoxicant and a parasite can promote within-host infection and reduce health status. The immune response of the host can be divided into three phases of increasing toxicant load; phase I, II and III (Figure 2). In the first phase, sub-lethal doses of the toxicant damage the immune system. This results in suppression of the immune system and hence the individual organism becomes highly infected. In the second phase, intermediate exposure to the toxicant reduces the total density of parasites. In the third phase, the extremely high exposure to the toxicant leads to the loss of within-host cells and eventual mortality of the host.

Through disentangling the individual effects of both lethal and sub-lethal toxicant exposure, we were able to establish the role of each within the breakdown of within-host dynamics. Indirect (sub-lethal) suppression of the immune system causes rapid proliferation of parasites within the host (Figure 3), while direct (lethal) mortality cause both parasites and within-host cells to die. However without the direct effect of the toxicant on the production of new cells, the host remains highly infective (Figure 4). We also predict

that an extremely small toxicant exposure can cause the proliferation of a previously manageable infection.

The findings we present in this study shed new light on the poorly understood mechanism by which toxicants seem to interact synergistically with infection to increase mortality risk [65]. In the context of the recent losses to honey bees populations [6, 45, 46], the synergistic immunotoxicant-infection interaction studied here is one example of the recent hypothesis that widespread honey bee losses may be multi-factorial [53, 54, 55, 56]. Synergistic pesticide-infection interactions have been shown to increase mortality risk within honey bees [34, 35]; for example, *Nosema ceranae* infections and thiacloprid, a neonicotinoid pesticide act synergistically to increase individual mortality [37]. The findings we present in this paper propose one explanation of how synergy between these toxicants and infection occur at the within-host level. We show that these sub-lethal effects of anthropogenic stress are potentially more damaging to individual health, aggravating parasitic stress. This is in direct agreement to the positive correlation between low level (field condition) neonicotinoid treatment and increases in parasite and viral infestations in bees [72, 73]. Infections within individual honey bees can be significantly increased by different levels of low or high sub-lethal pesticides [36]. Indeed, honey bees with undetectable levels of neonicotinoid imidacloprid which are reared in sub-lethal conditions still have increased infection levels [36]. This suggests that even extremely small sub-lethal exposure to pesticide can result in outbreaks of infection. We show that increasing the

pesticide exposure by a small amount ($Q > 0$) can result in a transition from a manageable parasite density level to a highly infected individual.

Our results rely upon condition (3) which ensures that the immune response is destroyed before the within-host cells. This condition is crucial to ensuring reasonable behaviour of the model, and it should be noted that the reverse assumption predicts the presence of immunity even after both infected and within-host cells are dead (Figure 5a). We highlight this limitation of our theoretical work but argue that condition (3) is valid since the direct lethality of toxicants only occur at high doses [18] and various immunosuppressive effects occur from toxicants [39], thus suggesting that toxicants have a greater impact on suppressing the immune system.

The framework provided in this study focuses on the failure of the immune system of an individual organism. However individuals interact within populations causing infection to spread to other susceptible individuals, and these populations have associated interdependent immune defences at both the within-host and between-host level. For example, social immunity involves many behavioural and population-level mechanisms such as social fever, a mechanism by which individuals increase the temperature of the surrounding environment in order to kill parasites [74], guarding, where patrolling guards prevent infected individuals from interacting with healthy individuals [75], hygienic cleaning behavioural traits, by which the population remove diseased or dead individuals [76] and storing antimicrobial food [77]. Hence the main limitation of our framework is that we may have only considered

one half of both interdependent within and between-host immunities. Coupling population immunity models in the context of an epidemic alongside our individual immunity framework could further explain the synergistic interactions between toxicants and infection at both the individual and population level. Further theoretical work incorporating these multi-level dynamics could address the gap in understanding honey bee sudden collapse as synergistic stressors in similar ways to other models of colony collapse disorder [78, 79, 80].

This work highlights the need for further studies which focus on synergistic interactions between various stressors at the within-host level. Our theoretical study presents a starting position to think about these synergistic interactions at the within-host level in the context of the immune system of an individual organism. While our model has an inherently simple structure, the addition of the toxicant function can lead to complicated dynamics that are consistent with empirical observations. This framework can stimulate further empirical and theoretical studies which focus on the interaction between toxicant exposure, infection and the immune system at both the social group and individual level.

5. Acknowledgments

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6. Competing Interests

We declare we have no competing interests.

7. Authors' Contributions

All authors conceived the idea for the study, constructed the model and analysed and interpreted the material. R.D.B. wrote the manuscript, with contributions from all authors.

8. Figures and Tables

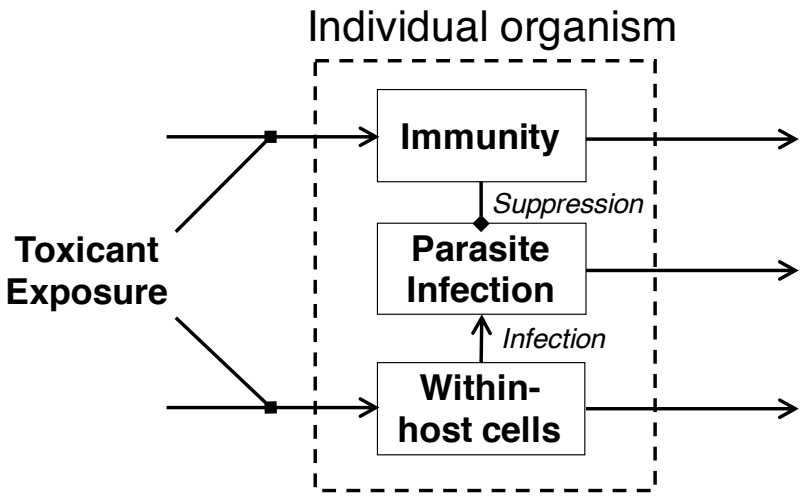
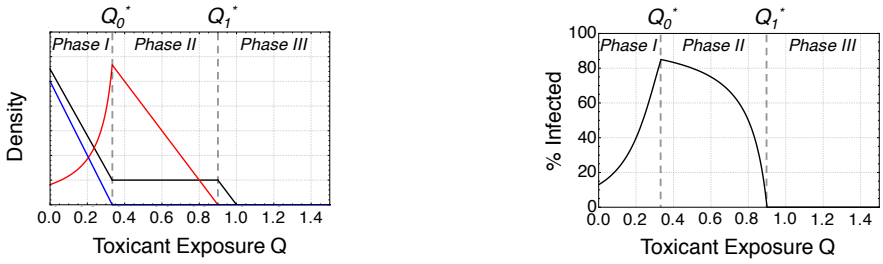
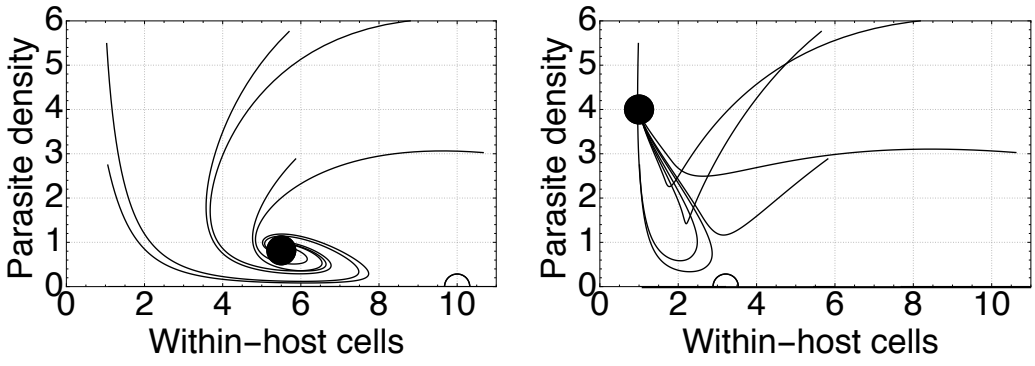


Figure 1: The modelling framework we use to model the interaction between toxicant exposure and parasite infection in an individual. Block arrows represent suppression. We model toxicant exposure as a suppressive effect on immunity and within-host cells.



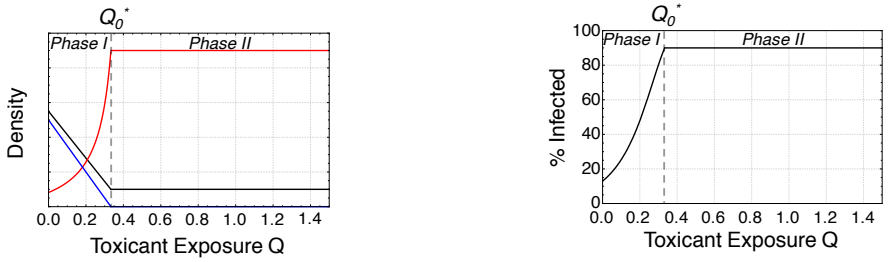
(a) (b)

Figure 2: The mechanism of parasite infection under increasing toxicant exposure. This shows the parameter dependence of immunity, parasite density and within-host cells at equilibrium within the dynamics of our model. In (a) the total densities of immune function (blue), parasite load (red) and within-host cells (black) change as an individual is subject to higher toxicant loads. In (b) the total % parasite infection (black) changes as the toxicant load is increased. Parameters as in Table 2.



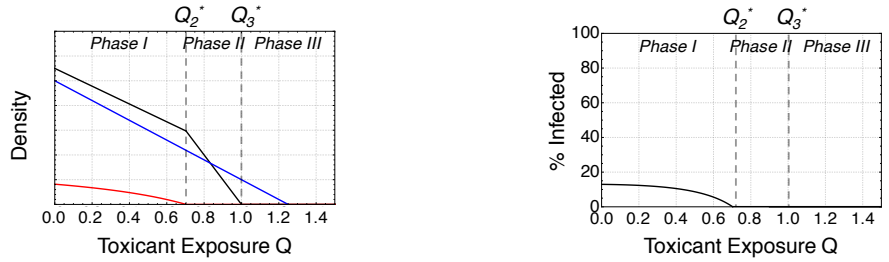
(a) Toxicant exposure $Q = 0$ (b) Toxicant exposure $Q = 0.5$

Figure 3: The convergence of the total density of within-host cells and parasites under no toxicant exposure (a) $Q = 0$, and small amounts of toxicant exposure (b) $Q = 0.5$. Black dots show the stable endemic equilibrium, white dots show the unstable disease-free equilibria and lines show the convergence from initial conditions. Parameters as in Table 2 and we assume an initial immune response ($Z = 10$) and an initial amount of within-host cells ($X > 0$), and either zero or positive parasite density ($Y \geq 0$).



(a) (b)

Figure 4: The mechanism of infection under increasing toxicant exposure, under only the immunosuppression of the toxicant effect. In (a), the total density of immune function (blue), parasite load (red) and within-host cells (black) change as an individual is subject to higher toxicant loads. In (b), the total % parasite infection (black) changes as the toxicant load is increased. Parameters as in Table 2.



(a) (b)

Figure 5: The mechanism of parasite infection under increasing toxicant exposure with aggressive direct mortality. In (a), the total density of immune function (blue), parasite load (red) and within-host cells (black) change as an individual honey bee is subject to higher toxicant loads. In (b), the total % parasite infection (black) changes as the toxicant load is increased. Parameters taken from Table 2, but with a reduced indirect effect $h = 0.08$.

	No parasite infection	Initial parasite infection
Within-host cells X	reduced by $\frac{rQ}{d}$	reduced by $\frac{hpQ}{b\beta}$
Parasites Y	no change	increased by $\frac{bQ(hp\lambda - abr - cpr)}{(ab+cp)(ab+p(c-hQ))}$
Immunity Z	reduced by $\frac{rQ}{d}$	reduced by $\frac{hQ}{b}$

Table 1: The net change of immunity, within-host cells and parasites after the introduction of toxicant, compared to the no-toxicant model, for both initial and no parasite infection load.

Parameter	Symbol	Value
production of within-host cells	λ	0.1
rate of parasite infection	β	0.01
death of within-host cells	d	0.01
direct lethal effect of toxicant	r	0.1
toxicant exposure	Q	$[0, 1.5]$
death rate of parasites	a	0.01
immune suppression	p	0.009
production of immunity	c	0.1
removal of immunity	b	0.02
indirect sub-lethal effect of toxicant	h	0.3

Table 2: The parameters used in the analysis of the model.

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