Verified models describing how agrochemicals affect bee health

Deliverable D8.2

31 August 2022

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PoshBee

Pan-european assessment, monitoring, and mitigation of stressors on the health of bees

Prepared under contract from the European Commission

Grant agreement No. 773921
EU Horizon 2020 Research and Innovation action

Project acronym:	**PoshBee**
Project full title:	**Pan-european assessment, monitoring, and mitigation of stressors on the health of bees**
Start of the project:	June 2018
Duration:	60 months
Project coordinator:	Professor Mark Brown Royal Holloway, University of London www.poshbee.eu

Deliverable title:	Verified models describing how agrochemicals affect bee health
Deliverable n°:	D8.2
Nature of the deliverable:	Report
Dissemination level:	Public
WP responsible:	WP8
Lead beneficiary:	SLU
Citation:	Annoscia, D., de Miranda, J.R., Topping, C.J., & Nazzi, F. (2022). *Verified models describing how agrochemicals affect bee health*. Deliverable D8.2 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921.
Due date of deliverable:	Month n° 51
Actual submission date:	Month n° 51

Deliverable status:

Version	Status	Date	Author(s)
1.0	Draft	1 August 2022	Name: D. Annoscia, J.R. de Miranda, C.J. Topping, F. Nazzi Organizations: UNIUD, SLU, AU
2.0	Final	31 August 2022	Name: D. Annoscia, J.R. de Miranda, C.J. Topping, F. Nazzi Organizations: UNIUD, SLU, AU

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Summary

In order to design, implement and evaluate possible management practices aimed at optimizing the health, well-being and survival of bees in different environments, one not only should be able to describe bee health but should also be able to predict bee health as affected by the possible perturbing factors. For this purpose, descriptive models are an invaluable tool; however, the lack of an exact quantitative knowledge of the many parameters influencing bee health at individual and colony levels poses a serious challenge to this approach. On the other hand, theoretical and computational tools are now available to assess the parameter-independent, structural properties of biological systems. The knowledge of these properties allows a mechanistic understanding of the processes underlying bee health and thus the capacity to predict its fate in the face of the many challenges affecting the well-being of bees.

Through system analysis here we show that studies assessing the effect of any single stressor, including insecticides, on honey bee health can generate multiple results. Subsequently confirmed by laboratory experiments, this effect is due to non-linear feedbacks. The resulting unpredictability is related to the complex nature of the system under study, and in particular to the immune-suppressive capacity of a widespread pathogen of honey bees. Based on the current knowledge about the immune system of bumble bees and wild bees, we speculate that the case of non *Apis* bees should be different and the effect of toxic chemicals on them more easily predicted.

1. Introduction

1.1. Bee health

In Deliverable D8.1 we presented a working definition of 'health' from the perspective of bees and identified a set of key indicators for measuring and evaluating the health status of bees. We regarded bee health as a hierarchical set of interdependent homeostatic layers, or systems that individually and together protect the bee against short-, medium- and long-term fluctuations in the environment. According to this definition, bee health is the result of a continuous "dialogue" between the environment and the homeostatic response of bees.

The number of environmental factors that can potentially affect bee health is huge but they can be grouped into a few categories, including: parasites and pathogens, agrochemicals, forage resource availability, and environmental conditions (Goulson et al., 2015). Agrochemicals have attracted considerable attention for their potential negative effects on pollinators; the case of neonicotinoids is particularly interesting because, despite the negative effect of these compounds having been clearly established in the laboratory, field testing has resulted in contradictory outcomes (Godfray et al., 2014). For example, no detectable negative effects were reported on honey bees maintained near Clothianidin-treated oilseed rape fields in some countries (Cutler et al., 2014; Rolke et al., 2016; Osterman et al., 2019), whereas in a large-scale experiment spanning three European countries, both negative and positive effects were noted (Woodcock et al., 2013). The lack of negative results observed in some cases has been attributed to the buffering capacity of honey bee colonies (Henry et al., 2015; Osterman et al., 2019). However, the reason why such buffering capacity could prevent apparent harm under certain conditions, but not others, remains unclear. The variability of the contexts where the studies were carried out, involving both the possible stress factors and the quantity-quality of available nutrition, certainly plays a role, but this plausible explanation lacks the necessary robustness in a case whereby absence of evidence has often been regarded as evidence of absence. Overall, it appears that our capacity to interpret the complexity of the situations that bees can encounter in the field is seriously limited.

In Deliverable D8.1 we identified several homeostatic layers maintaining bee health in the face of the possible fluctuations of the environmental factors listed above, including: molecular, cellular, organismal, individual, social, ecological, and evolutionary. If we restrict our attention to individual bee health, the molecular, cellular and organismal levels are the most relevant and, in particular, the control systems responsible for immunity, detoxification and metabolism.

1.2. "Describing" bee health vs "predicting" bee health

Bee health can be described and even be measured using a set of appropriate indicators, here intended as an estimate of a given health dimension in a target population. Such indicators are quantitative estimates of a particular trait in the bee or its environment that can be predictably linked to a certain aspect of health. Several indicators that can be used to describe bee health were listed in Deliverable D8.1.

However, in order to design, implement and evaluate possible management practices aimed at optimizing the health, well-being and survival of bees in different environments, one not only should be able to describe bee health but should also be able to predict bee health as affected by the possible perturbing factors.

Descriptive models could help in drawing more or less accurate predictions regardless of the inevitable variability of contexts (Mickaël et al., 2017) and thus support risk assessment and the consequent decisions (Topping et al., 2020) as well as the design of management practices. Indeed, deliverable D8.3 is a descriptive model of a bumble bee colony that will complement similar models regarding the honey bee and *Osmia* that are being developed outside Poshbee. These models represent a significant advance towards the possibility of predicting bee health of a few representative species as affected by several environmental factors, including agrochemicals. However, the lack of an exact quantitative knowledge of the many parameters influencing bee health at individual and colony levels still poses a serious challenge to this approach.

On the other hand, theoretical and computational tools are now available to assess the parameter-independent, structural properties of biological systems (Giordano et al., 2016; Blanchini & Giordano, 2021). The knowledge of these properties allows a mechanistic understanding of the processes underlying bee health and thus the capacity to predict its fate in the face of the many challenges affecting the well-being of bees.

This systems biology approach is based on a detailed knowledge of the biological system to be modelled and a convenient representation of it by means of an adequate conceptual model, followed by the study of its structural properties. These are properties that exclusively rely on the architecture of the system under study and are independent of the strength, often unknown, of each single interaction. Structural approaches can provide qualitative insight into complex webs of interactions, even in the absence of knowledge about parameter values, and unravel the synergistic net effect of multiple stressors on bee health.

Through these methods we showed, first in theory and then in vivo, how the impact of toxic compounds on honey bee health can be shaped by the concurrent stressors affecting bees, eventually leading to multiple outcomes depending on initial conditions (this part of the analysis is currently under consideration for publication in a scientific journal (Breda et al., submitted). Following this we report the main conclusions of the paper and refer the reader to it for more details on the methods).

Subsequently we considered the case of other bees (i.e. the bumble bee *Bombus terrestris* and the red mason bee *Osmia bicornis*) to underline the possible significant differences with the honey bee as a first step towards the development of similar models for these species too.

2. A conceptual model of honey bee health

2.1. The model

Based on available data derived from the literature, the work carried out within other work packages of this project, and our own experiments, a conceptual model of stressors and drivers potentially affecting honey bee health was built (Fig. 1). This model describes the health of honey bees as influenced by multiple stress factors and effects including: (a) ectoparasites such as the mite *Varroa destructor* (Nazzi & Le Conte, 2016), (b) viral pathogens like the Deformed wing virus (DWV) (Grozinger & Flenniken, 2019), (c) toxic compounds (Johnson, 2015) and adverse environmental factors, in particular (d) sub-optimal thermal conditions (Bernd, 1981). Sugars from nectar (e) and pollen (f) are used by bees as a source of energy and protein and promote honey bee health (Brodschneider & Crailsheim, 2010), but can be contaminated with toxic compounds (g, h) (Chauzat

et al., 2006; Mitchell et al., 2017). Honey bees invoke a number of mechanisms to combat stress factors; in particular, an immune response is normally activated to combat parasites (i) (Annoscia et al., 2020) and pathogens (j) (Brutscher et al., 2015), and a detoxification system (k) can reduce the concentration of toxic compounds (Berenbaum & Johnson, 2015). Individual reactions, such as increased sugar feeding (l), can be used to counteract low temperatures (Bujok et al., 2002) and this may even expose bees to higher contamination of toxic compounds. Some of the factors themselves can influence the honey bee homeostatic responses; in particular, DWV has the capacity to impair the immune response (m) (Nazzi et al., 2012), which can likewise be reduced by some toxic compounds (n) (Di Prisco et al., 2013). Mite infested honey bees may consume less sugar (o) (Frizzera et al., 2021). Stressors can also interact and a potential negative effect of lower temperatures on the parasite cannot be excluded (p) (Le Conte et al., 1990).

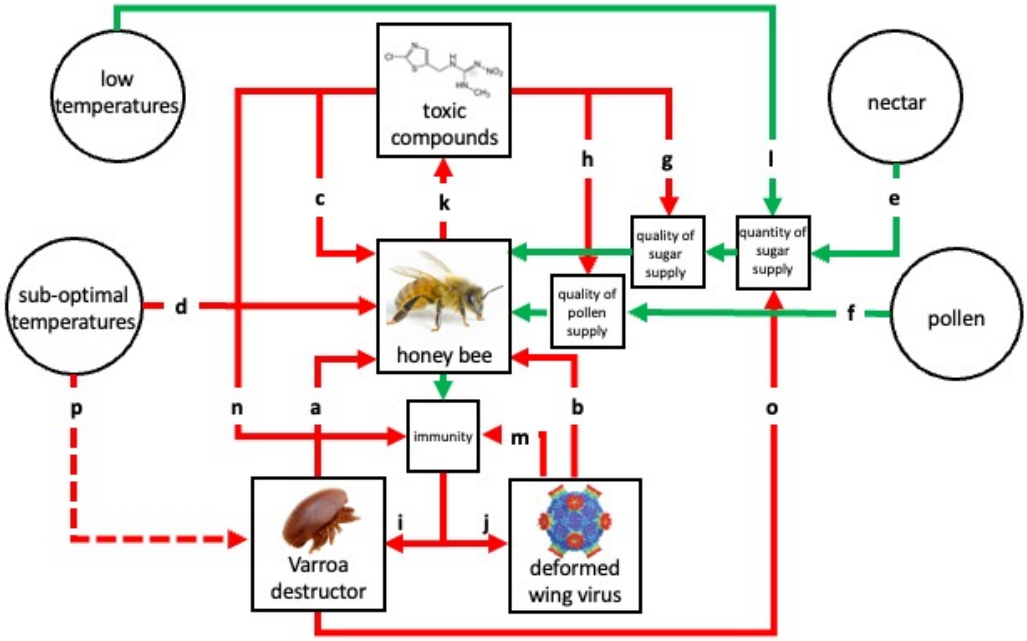

Figure 1 A conceptual model of honey bee health. Red and green arrows represent negative and positive interactions, respectively (e.g. red arrow "c" represents the negative effect that toxic compounds can exert on honey bee health; green arrow "f" represents the positive effect of pollen on honey bee health).

Many more stressors, including more than twenty viruses, a plethora of toxic substances, several parasites and a countless combination of environmental factors may influence bee survival (Goulson et al., 2015). However, as far as our analysis is concerned, the proposed representation of the system already captures all the relevant qualitative interactions, irrespective of the specific identity of the stressors involved and the quantitative details. For example, we included just one toxic compound, even though many pesticides can impact honey bees at the same time (Mullin et al., 2010) and toxic compounds can interact with one another, as in the case of fungicides increasing the toxicity of insecticides (Pilling & Jepson, 1993; Sgolastra et al., 2017; Wernecke et al., 2019). Our

model may thus be seen as an oversimplification of the system under study, and it would be so if our objective were to derive a descriptive model aiming at quantifying bee health at any given time, in the presence of a defined amount of certain stressors. However, for the purpose of the structural analysis we carried out, the case of one toxic compound exerting a negative effect, or that of more toxic compounds interacting with one another so as to exert an even bigger negative effect on honey bees, are equivalent, because the sign of the resulting effect is the same.

According to our conceptual model, the dynamic interplay between honey bee health and the surrounding environment can be described by a system of ordinary differential equations representing the interactions among the key components (variables) in our conceptual model (Fig. 2).

$$\tau_{HB}\dot{x}_{HB} = -\delta_{HB}x_{HB} + g_{TC}(x_{TC}) + g_{VA}(x_{VA}) + g_{VI}(x_{VI}) + \overline{f}_{S,C}(u_S, u_C, x_{TC}, x_{VA}) + \overline{f}_P(u_P, x_{TC}) + \underline{f}_{HB}(u_T)$$
$$\tau_{TC}\dot{x}_{TC} = -\delta_{TC}x_{TC} + g_{HB}(x_{HB})$$
$$\tau_{VA}\dot{x}_{VA} = -\delta_{VA}x_{VA} + h_{VA}(x_{HB}, x_{TC}, \varepsilon x_{VI}) + \underline{f}_{VA}(u_T)$$
$$\tau_{VI}\dot{x}_{VI} = -\delta_{VI}x_{VI} + h_{VI}(x_{HB}, x_{TC}, \varepsilon x_{VI})$$

Figure 2 The system of differential equations describing the model depicted in Fig. 1. x_{HB} represents honey bee health, x_{TC} the stress due to toxic compounds (e.g., neonicotinoid insecticides), x_{VA} the stress due to parasites (e.g., *Varroa destructor*) and x_{VI} the stress due to pathogens (e.g., DWV). The system includes the effects of the external inputs: sugar u_S, pollen u_P, absolute deviation from desired temperature u_T and suboptimal temperature u_C. The coefficients τ denote the time constants, δ denote the "self-control" of each key-player.

In particular, the first equation shows that honey bee health (x_{HB}), which is self-regulated by internal physiological mechanisms described by δ_{HB}, can be negatively influenced by toxic compounds (x_{TC}), parasites (x_{VA}) and pathogens (x_{VI}), according to various mechanisms described by different monotonically decreasing functions (i.e., g_{TC}, g_{VA}, g_{VI}) because each factor exerts a negative effect on honey bee health, as denoted with the common symbol g. Similarly, honey bee health is affected by other factors (e.g., nutrition, represented by the external inputs u_S and u_P; sub-optimal temperatures u_T and low temperatures u_C), whose influence can be modified by other stress factors (e.g., toxic compounds that can contaminate food stuff). These interactions are represented by functions that are increasing in the case of favorable influences and decreasing in the case of adverse effects.

2.2. Structural analysis of the bee health model

The structure of the system under study was analyzed using the community matrix concept (Breda et al., submitted); this describes the interactions among the components of an ecological system near equilibrium (Levins, 1968). This analysis revealed that the system is monotone (Hirsch & Smith, 2006), meaning that the ordering of solutions with respect to initial data is preserved. When this is the case, despite the possible intricacies, some important features of the system dynamics can be inferred on the basis of purely qualitative or relatively basic quantitative knowledge of the system characteristics (Angeli & Sontag, 2003; Sontag, 2007).

The effect of an external input applied to the system variables on the steady-state variation of each of the others can be represented by a structural influence matrix (Giordano et al., 2016) reporting the net effects, including both direct and indirect effects of a stressor on the others (Fig. 3; for methodological details on the matrix computation see: Breda et al., submitted).

Influence of	HB	TC	VA	VI
on HB	+	-	-	-
on TC	-	+	+	+
on VA	-	+	+	+
on VI	-	+	+	+

Figure 3 The structural influence matrix of the system, where the term in position (i,j) represents the parameter-independent sign (positive, negative, or zero) of the variation of the steady state of key player i ensuing from the application of a constant input affecting key player j; this can be seen as the net effect of j on i, including both direct and indirect effects. HB, TC, VA, VI are honey bee health, toxic compounds, parasites, viruses, respectively.

Unsurprisingly, the structural influence matrix shows that any new stressor applied to the system has a net negative effect on bee health. Thus, a toxic compound, such as for example a neonicotinoid insecticide, can only have a negative effect on honey bee health when applied to individual bees, regardless of the presence of parasites and pathogens. Hence, the lack of a detectable effect reported in some cases could naively be regarded as a lack of the hypothesized detrimental effect. However, this observation is superficial and does not consider the complexity of the system, as clearly revealed by a detailed study of the system equilibria.

2.3. System equilibria

Although an analytical solution of the differential equations representing our biological system, and thus the calculation of each variable at each time, is not possible, the study of the equilibria of the system can explain its behaviour under different conditions.

Equilibria are the simplest solutions of the dynamical system representing honey bee health as affected by stressors and drivers and represents the value of the state variables (e.g., x_{HB}, representing honey bee health) where they do not change, or, in other words, the possible destiny of a variable provided it is allowed to (and can) settle to a constant value. Therefore, the study of system equilibria can discriminate whether honey bee health, represented by the first equation in Fig. 2, can settle to a high, satisfactory level, or is bound to deteriorate to a lower, dangerous level, when insects are exposed to a certain set of stressors.

In order to provide a visual description of our results, we specified the form of each function and assumed a set of values for the model parameters, then we plotted the orbits and the equilibria on the projected phase planes (Breda et al., submitted). In this way we could graphically describe the trajectory of each variable with respect to others; in particular, we could see how honey bee health reacts to increasing pressure of viruses, parasites or toxic compounds and the end point of this process. Please note that our arbitrary selection of parameters (which are highly uncertain) does not influence the general qualitative conclusions of this study.

To investigate stability in the presence of different stressors, we exploited a reasonable and quite general representation of the system. In particular, we considered two alternative cases: the first of a pathogen that cannot influence the immune response of honey bees; the second, where the viral pathogen can affect the honey bee's immune system, as in the case of DWV (Nazzi et al., 2012).

In the first case, after appropriate mathematical treatment (reported in Breda et al., submitted), we found that the system admits a unique positive equilibrium, which is globally asymptotically stable in the positive orthant, whereby the position of the equilibrium on the honey bee axis depends on the intensity of the stressors or their combination (Figs. 4A, 4B). In particular, in the presence of a pathogen that cannot impair immunity, honey bee health is high when the level of parasites (or any other stressor) is low (Fig. 4A), and vice versa (Fig. 4B).

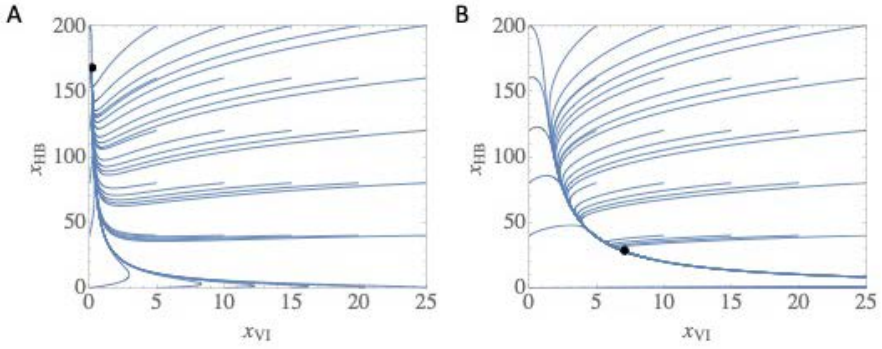

Figure 4 Some orbits (lines) and equilibria (black dots) of the full system in the projected phase plane of honey bee health (x_{HB}) and level of viral infection (x_{VI}). The equilibria and the values that the state variables can assume while approaching equilibria are represented with black dots and orbits (blue lines), respectively. (A) Orbits and the unique equilibrium without immune suppression, in the presence of a low level of parasites. (B) Orbits and the unique equilibrium without immune suppression, in the case of a high level of parasites. Each dot making up the blue curves (orbits) represents a possible starting condition in terms of heath, viral infection, parasite pressure and contamination with toxic chemicals; the shape of the curves, all converging on the black dots, show that, without immune-suppression, whatever the starting condition, honey bee health will settle to a high (A) or a low level (B) according to the pressure of the stressors.

In other words, it appears that, in the presence of a stable input of the stressors included in our model, honey bee health sets at a certain level. If either the level of parasite or pathogen pressure or pesticide contamination or both is too high, this equilibrium can be unbearable by the individual bee, resulting in death. In any case, the result can be predicted with a good degree of confidence based on the initial conditions; in fact, global stability makes the result independent of the initial conditions, as highlighted by the orbits in Figures 4A and B that are converging on the same equilibrium point (represented by the black dots in the figures) from different initial conditions (represented by any point on the blue lines in the figures).

We then considered the presence of a pathogen with the capacity to affect the immune response of honey bees. In this case, a convenient mathematical treatment (reported in Breda et al., submitted), relying on bifurcation theory (Kuznetsov, 1988), reveals a completely different scenario: the system can now support three equilibria, one of which is unstable, and hence bistability arises (Fig. 5).

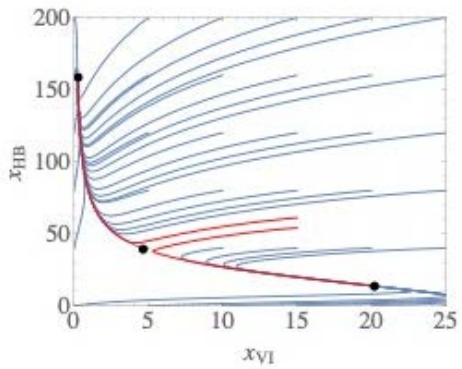

Figure 5 Some orbits (lines) and equilibria (black dots) of the full system in the projected phase plane of honey bee health (x_{HB}) and level of viral infection (x_{VI}). Orbits and the three equilibria with immune suppression; two orbits exiting from close initial conditions are marked in red. Each dot making up the blue curves (orbits) represents a possible starting condition in terms of heath, viral infection, parasite pressure and contamination with toxic chemicals; all curves above the upper red line converge on the black dot located in the upper left corner of the phase plane, while those below the lower red line converge on the bottom right black dot, meaning that, in case of immune-suppression, bees with similar health conditions (i.e. just above the upper red line, or just below the lower red line, right of the central black dot, representing the unstable equilibrium) may settle either to a high (A) or to a low health level (B), depending on minimal differences in their initial conditions.

In practice, under reasonable and biologically meaningful conditions, if the immune suppression capacity is absent or low, a unique stable equilibrium exists in the range of high bee health. For higher immune-suppression (i.e., larger values of the crucial parameter ε in the third and fourth equations in Fig. 2) a fold bifurcation (Kuznetsov, 1988) creates two additional equilibria. Of the resulting three equilibria, two are stable and are located in the high and low bee health regions, respectively. Increasing ε further moves the intermediate unstable equilibrium towards the high bee health stable one, until they collapse and disappear through a second fold bifurcation, leaving just one stable equilibrium in the low bee health region, when the immune suppression capacity is too large.

In fact, the addition of a pathogen that is capable of interfering with the immune response corresponds to the introduction of a critical positive feedback loop into the system (formed by arrows “m” and “j” between “immunity” and “deformed wing virus” in Fig. 1). Indeed, the higher the viral load, the stronger the suppression of the immune system, and the lower the efficiency of the latter to contain the virus, which can then actively replicate leading to higher viral loads.

In conclusion, the introduction of a pathogen capable of interfering with the honey bee's immune system generates an unstable intermediate 'watershed' equilibrium, which explains why, in the presence of slightly different initial conditions, vastly different outcomes can be possible (see red curves in Fig. 5).

Under more descriptive terms, if a stressor is above a certain level, there is only one equilibrium at low bee health, meaning for example that if a toxic compound is present at a harmful concentration, bee survival will be significantly lower, and a negative effect will be noted; instead, if the same stressor is below that dangerous level, one equilibrium at high bee health is certainly possible; meaning that, if the toxic compound is present at a low concentration, bee survival may not be significantly different from normal and a negative effect may not be noted. Interestingly, our analysis revealed that, in the presence of an immune suppressing virus, bistability can occur so that, for the same intermediate level of one stressor, one can have either low bee health or high bee health depending on the similar, but not identical, initial conditions and therefore the results may become unpredictable. In other words, in the presence of an intermediate amount of a toxic compound, a virus infected bee can either die prematurely or survive much longer, depending on its initial, intrinsic individual situation.

2.4. Validation of the bee health model

To experimentally test the predictions of our mathematical analysis showing bistability, we used data from several survival experiments, carried out in our lab using the same standardized method, over a six year period (for more details on the methods and the experimental date, see: Breda et al., submitted).

In this case, to test our theoretical predictions we used the longevity of caged bees as an estimator of their health condition and exploited the seasonality of a common immune-suppressing pathogen to determine the effects of its presence/absence on the longevity of honey bees. We hypothesized that, in the presence of an immune-suppressing pathogen (a condition normally occurring late in the season, in the area where the experiments were carried out (Nazzi et al., 2012)), the predicted bistability should result in bees at high bee health dying later in life and bees at low bee health dying earlier in life, with a consequent increase in the variability of longevity data.

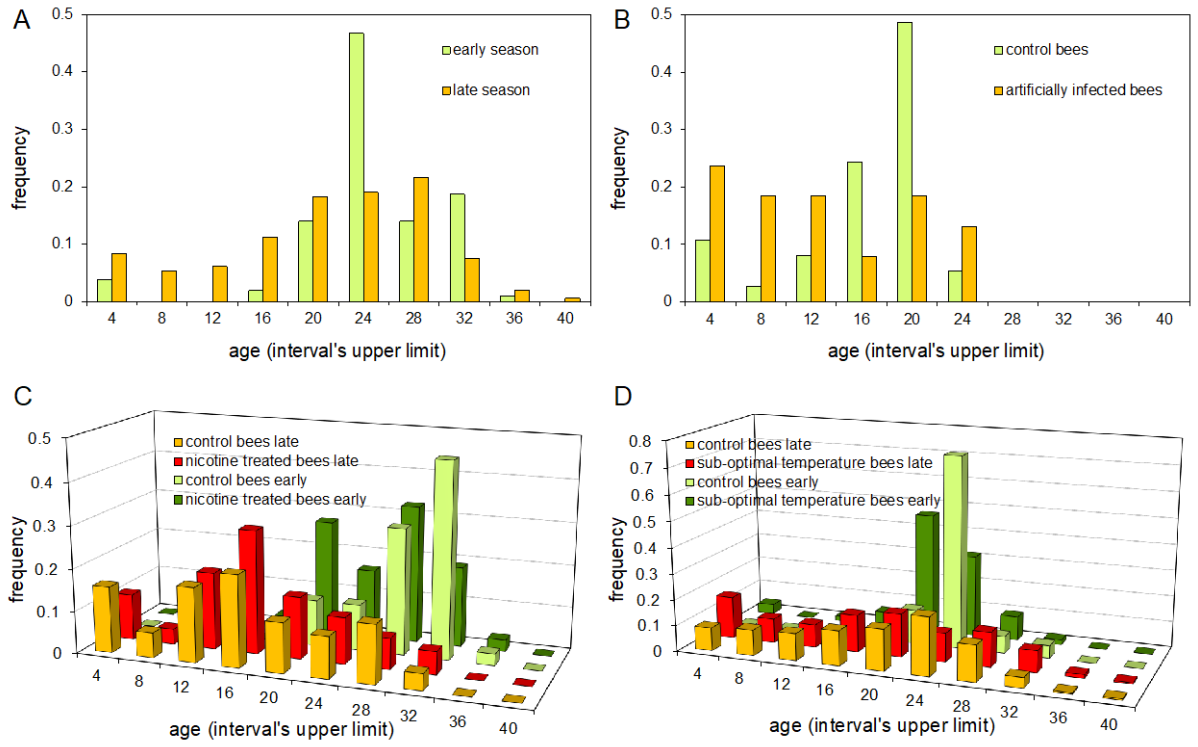

Figure 6 Distribution of individual lifespans of honey bees under different conditions. (A) Early in the season when the prevalence of an immune-suppressing virus is low (light green bars) and later when all bees are virus infected (orange bars). (B) Treated or not (orange and light green bars, respectively) with a virus administered to mature larvae through the diet. (C) When exposed to a toxic compound, when the prevalence of an immune-suppressing virus is low (green bars) or when the virus is widespread (red bars); the corresponding distribution of honey bees sampled early or late in the season and not exposed to the toxic compound as a control (light green and orange bars, respectively). (D) as (C) but exposed to a sub-optimal temperature in place of a toxin.

We first tested the effect that the addition of an immune-suppressing virus has on the survival of caged honey bees. To this aim we compared the survival of bees maintained under the same conditions and sampled either early in the season, when the prevalence of a known immune-suppressing virus (i.e., DWV) is low and late in the season, when most bees are DWV infected, as confirmed by subsequent molecular analysis. Virus free honey bees from early year populations had a characteristic survival curve with limited mortality during the first three weeks of life, followed by another two weeks of increased mortality and a distribution of lifespans centered around 23 days of age (Fig. 6A). Instead, virus infected honey bees from late year populations showed a much broader distribution of lifespans, with a significant number of bees dying at a young age and others surviving much longer (Fig. 6A). As a result, the interquartile range of longevities, here used as a measure of the dispersion of data, was 6 in early year bees and 10 in late year populations, indicating a higher variability of longevity data in the presence of an immune-suppressing virus.

In a second experiment, virus free honey bees, collected early in the season, were artificially fed virus particles and the tests repeated, confirming the results reported above (Fig. 6B). In particular, we found that control bees had a median longevity of 18 days and an interquartile range of 5, whereas virus treated bees had a much shorter median longevity (i.e., 10) as a result of a large

number of bees dying in the first days, as underlined by a much larger dispersion of longevity data (interquartile range = 12). This further supports the view that the presence of an immune-suppressing virus can create vastly different outcomes depending on the slightly different initial conditions of single bees exposed to otherwise identical situation.

In summary, by carrying out two different comparisons of uninfected versus virus infected bees (one diachronic, with naturally virus infected bees sampled at two different times, and one synchronic, by treating or not with the virus some uninfected bees at the same time), we noted that uninfected bees show a mortality concentrated after three weeks of life, as expected in view of the shape of the survival curve of control caged bees previously observed under the same conditions (Annoscia et al., 2017). In contrast, the mortality of virus infected bees is not concentrated late in life but can also occur at a young age, resulting in a marked variability of longevities. Thus, as predicted by our model analysis, the probability of dying either soon or late does not only depend on the treatment but rather on the slightly different intrinsic conditions of bees. These were not under our control, but nonetheless dictated the bee's final destiny.

To investigate how the presence of an immune-suppressing virus could alter the response of honey bees to different stressors, we carried out two more experiments, whereby we studied the survival of honey bees exposed to 50 ppm of nicotine, here used as an example of a toxic compound, or to the sub-optimal temperature of 32 °C, as compared to the normal in-hive temperature of 34.5 °C (Bernd, 1981).

When the virus was not present, both stressors caused a decreased lifespan, showing a distribution of lifespans shifted towards shorter ages (Figs. 6C and 6D, light and dark green bars). However, in presence of a virus, both in the case of a toxic compound and a low temperature, a much broader survival distribution was generated, consistent with the bistability hypothesis (Figs. 6C and 6D, orange and red bars). Accordingly, the interquartile range of longevities increased from values from 3 to 7 in early year populations, to values from 8 to 18 in late year populations, highlighting a higher variability of longevity data, both in case of a toxic compound and a low temperature.

Overall, these results show that the presence of a pathogen capable of interfering with immune-control creates a situation whereby the survival of honey bees is not solely determined by the external stressors. Rather, it is profoundly influenced by some minimal variations in the starting conditions, leading either to an imbalanced condition and premature death (lower red orbit in Fig. 5), or coping with the stress much longer (upper red orbit in Fig. 5).

3. The case of other bees

Both the structural analysis we carried out and the study of the system's equilibria are based on a schematic representation of the various factors affecting honey bee health. As far as the structural analysis of the system is concerned, neither the precise identity of the stressors involved, nor the strength of the interactions are as important as their respective sign (i.e. their positive or negative nature). In other words, it is the architecture of the systems that determines its behaviour, and, after all, the destiny of the variable called bee health. Under more practical terms, it does not really matter what chemical compound occupies the square labelled "toxic compound" in the conceptual model, nor does it matter if the negative effect of that compound, represented by the red arrow "c", is very strong or weak. Also, the case of a single red arrow connecting one toxic compound to bee

health or many red arrows converging to bee health from as many different toxic compounds is not different provided that the sign of the interactions is the same (i.e. if the effect is negative in all cases). This has important implications for the extrapolation of the conclusions that were drawn for honey bees to the case of other bees.

Since the kind of stressors potentially affecting bee health are the same for all bees, irrespective of their specific identity, there is no reason to think that bumble bee health or the health of a wild bee will be affected by the same stressors in a qualitatively different manner. For this reason, the possible conclusions regarding these other biological systems should be similar to those reported above for the honey bee, apart from one important detail. Specifically, to our knowledge, no parasite affecting the health of both bumble bees and wild bees has been shown to be capable of disrupting their immune system. This has important implications, because the capacity of DWV to impair the immune defence of honey bees appears to be the critical factor determining bistability and thus the fact that bees exposed to similar conditions can have marked different destinies depending on very subtle differences in their intrinsic conditions, which in turn is responsible for the limited predictability of the fate of honey bees exposed to intermediate levels of toxic chemicals. For this reason, we speculate that the health of both bumble bee and solitary bees, as affected by similarly dangerous toxic chemicals, should be more predictable than that of honey bees, notwithstanding the importance of sociality for buffering the negative effect of agrochemicals on bees.

Available data on the effect of neonicotinoid insecticides on both bumblebees and solitary bees under field realistic conditions (see for example: Woodcock et al., 2013; Osterman et al., 2019) seem to support our conclusions which, however, should be appropriately tested with dedicated experiments.

4. Conclusions

It is widely acknowledged that agricultural systems function as complex systems; within these, agrochemicals are an important component and can represent important threats for bees and the pollination service they provide to the benefit of crop production and biodiversity (for the case of neonicotinoids, see: van der Sluijs et al., 2013). This concern is based on a large and consistent body of evidence that was largely built under laboratory conditions, whereas studies carried out under field conditions have not provided similarly convincing data, generating uncertainty about the real risk posed by some substances under more realistic settings (for the case of neonicotinoids, see: Godfray et al., 2015).

By using our verified model describing how agrochemicals and other stressors affect bee health, we demonstrated in theory, and also confirmed in practice, that the already reported capacity of a widespread virus to impair the immune defences of honey bees can generate bistability. This implies that honey bees under similar initial conditions can have markedly different destinies when exposed to the same stressor.

It is important to underline that only the immune-suppressing pathogen can cause the bistability and the described dynamics, because of its capacity to attack the bee defense system, thus exacerbating its own effect (Nazzi et al., 2012). To our knowledge, no other stress factor can impair the system keeping that stressor under control, and thus be implicated in similar dynamics. In some cases an effect of pesticides on the detoxification system of honey bees has been reported (Boncristiani et al.,

2012). This is normally expressed as an up-regulation of some genes after exposure to pesticides (Derecka et al., 2013; De Smet et al., 2017; Zhu et al., 2017), likely indicating the activation of a pathway in response to intoxication. This does not suggest the capacity of that pesticide to impair detoxification, but should rather be regarded as evidence of a well-functioning homeostatic system that reacts to intoxication through a physiological mechanism aimed at reducing the concentration of the toxic chemical. However, based on our analysis we can hypothesize that a pesticide exhibiting the reversed effect (i.e., anti-detoxification), could cause system behaviour like that reported here for the pathogenic virus DWV. At present this possibility is purely speculative, but it may have important implications for honey bee survival should this type of pesticide be used in the future.

Our data allow a retrospective evaluation of published studies that may explain the contrasting results reported therein (Woodcock et al., 2013; Cutler et al., 2014; Rolke et al., 2016; Osterman et al., 2019). Based on our conclusions we hypothesize that, in the presence of a low prevalence of the immune-suppressing virus, the negative effect of pesticides at field realistic concentrations can be buffered by the colony's homeostatic response as previously proposed (Henry et al., 2015; Osterman et al., 2019), provided that other stressor effects are limited. In contrast, when the immune-suppressive virus or the vector mite is present, negative effects are more likely to be noticed because of the bistability we demonstrated, that may cause some bees to experience premature mortality which cannot be effectively buffered by the homeostatic response mechanisms of the colony.

Overall, this analysis demonstrates that considering relationships between components, rather than focusing on the individual, context-dependent, expression of a system state, leads to a deeper understanding and is a better basis for real world decisions. In fact, the bee system described here is a good example of the kind of feedbacks found in ecology and biology and is not unique. In cases like this, empirical observations of a single system state in space and time are important but have poor predictive power compared to the system analysis presented here.

5. Acknowledgements

We gratefully acknowledge: Dimitri Breda, Davide Frizzera, Giulia Giordano, Elisa Seffin, Virginia Zanni and Franco Blanchini for their contribution to the work leading to the conclusion reported here.

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