Hazard/Risk Assessment

USING BEEHAVE TO EXPLORE PESTICIDE PROTECTION GOALS FOR EUROPEAN HONEYBEE (APIS MELIFERA L.) WORKER LOSSES AT DIFFERENT FORAGE QUALITIES

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(Submitted 1 December 2015; Returned for Revision 1 February 2016; Accepted 19 May 2016)

Abstract: Losses of honeybee colonies are intensively debated and although honeybees suffer multiple stressors, the main focus has been on pesticides. As a result, the European Food Safety Authority (EFSA) revised the guidance for pesticide risk assessment for honeybees. The European Food Safety Authority reported a protection goal of negligible effect at 7% of colony size and then used the Khoury honeybee colony model to set trigger values for forager losses. However, the Khoury model is very simplistic and simulates colonies in an idealized state. In the present study, the authors demonstrate how a more realistic published honeybee model, BEEHAVE, with a few simple changes, can be used to explore pesticide risks. The results show that forage availability interacts with pesticide-induced worker losses, and colony resilience increases with forage quality. Adding alternative unexposed forage to the landscape also substantially mitigates the effects of pesticide exposure. The results indicate that EFSA’s reported protection goal of 7% of colony size and triggers for daily worker losses are overly conservative. The authors conclude that forage availability is critical for colony resilience and that with adequate forage the colonies are resilient to even high levels of worker losses. However, the authors recommend setting protection goals using suboptimal forage conditions to ensure conservatism and for such suboptimal forage, a total of 20% reduction in colony size was safe. Environ Toxicol Chem 2017;36:254–264. © 2016 The Authors. Environmental Toxicology and Chemistry published by Wiley Periodicals, Inc. on behalf of SETAC

Keywords: Pesticide risk assessment Population modeling Landscape ecology Population-level effects Mechanistic effect model Pollinator

INTRODUCTION

Reports of overwintering losses of European honeybee (Apis mellifera L.) colonies have increased over recent decades, and the search for the causes has been intensive [1–3]. Honeybees are exposed to multiple stressors such as Varroa mites (Varroa destructor) and the viruses they vector [4], lack of forage [5], Nosema, and other pathogens, and this has been compounded by changes to beekeeping practices and the profitability of beekeeping [1,6–11]. Although most agree that this cumulative load of multiple stressors underlies problems in honeybee colonies, much of the public and scientific debate has centered on the role of a single factor, namely, pesticides and in particular neonicotinoids [7,12], and the debate has been heated and at times emotional [13]. While several laboratory and semifield studies have indeed shown that high doses of neonicotinoids may affect survival or cause sublethal effects in honeybees (restated in Godfray et al. [12]), there is disagreement about the realism of the exposure levels used in these studies [12,14].

Neither widespread worker mortality nor colony losses have been confirmed in field experiments studying the effects of neonicotinoids [12,15–17]. However, exposure of honeybees following use of pesticides in the field is difficult to quantify because honeybees are social insects, and the colony acts as a superorganism whose different castes, and individuals within the castes, may experience very different exposures [18]. Honeybee colonies exploit numerous foraging sources over large spatial scales and respond rapidly to changing nectar and pollen availability [19,20], in-hive workers mix pollen and nectar from different sources [21], and residues dissipate through metabolism and decline during storage and handling [22]. Moreover, the colony has several sophisticated mechanisms that enable it to deal with changing forage availability and other stressors, such as changes to age of first foraging, cannibalism of brood during pollen shortages, and flexible egg-laying rate by the queen, making it difficult to predict how effects on individuals will impact colony dynamics [23–26]. Although it has not been explicitly stated, it appears that an underlying concern is that the complex colony dynamics of honeybees may result in the effects of exposure to systemic pesticides building up without being noticed, leading to colony loss occurring seemingly without warning. For instance, in a study on disorientation of foragers following exposure to a neonicotinoid, Henry et al. [27] used a simplistic honeybee colony model developed by Khoury et al. [28] and predicted widespread colony losses as a consequence of forager losses.

As a result of the conflicting evidence and these uncertainties about true levels of exposure, effects on individual bees, and the resulting colony-level impact, the European Food Safety Authority (EFSA) changed the guidance document for regulatory bee risk assessment of pesticides [29]. Of particular relevance to the present study are the changes to protection goals, trigger values for forager losses, and updated exposure calculation methods [29]. For higher-tier risk assessments and field studies, EFSA reports a protection goal of negligible effect on the colony as a 7% reduction in colony size [29], but the evidence behind this figure is unclear. Subsequently, EFSA used the same colony model as Henry et al. [27] (the Khoury et al. model [28]), to deduce what level and duration of forager losses would cause less than the protection goal of 7% overall decline in colony size and these values are then used to define trigger values [29]. However, the Khoury et al. [28] model excludes
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many of the mechanisms and feedback loops that allow honeybee colonies to exploit changing forage availability and cope with stressors; for example, neither honey stores nor seasonality are included [25,28].

Since then, a new realistic honeybee colony model, BEEHAVE, has been published by Becher et al. [30]. The BEEHAVE model combines what happens inside and outside the hive by simulating honeybee colony dynamics and agent-based foraging of nectar and pollen in a seasonal landscape. The European Food Safety Authority has reviewed BEEHAVE and concluded that, overall, BEEHAVE performs well in modeling honeybee colony dynamics, but that BEEHAVE is not yet usable in a regulatory context mainly because it lacks pesticide module and representative scenarios [31]. In the present study we demonstrate how BEEHAVE, with a few simple changes, can be used to explore protection goals and how scenarios for different weather patterns and landscape structures can be developed. We use BEEHAVE to explore the following questions: First, what are the forage requirements of colonies, and do these boundaries for survival have implications for pesticide exposure estimation? Second, how does forage quality affect colony resilience to worker losses? For this second question, the worker losses were simulated in 2 different ways to mimic 2 types of studies: colony assessment [32,33] studies and radiofrequency identification (RFID) transponder studies, which measures marked foragers return to hive [34]. Third, can alternative unexposed forage mitigate the effects of pesticide exposure? Finally, what level of worker losses is safe and could be used as protection goal under realistic worst-case conditions?

MATERIALS AND METHODS

The BEEHAVE honeybee model was used in all simulations. The model was developed by Becher et al. [30] and integrates processes inside the hive with landscape dynamics via a forager module. It was developed to study the impact of multiple stressors and is based on a review of existing models and existing knowledge of honeybees [25]. The BEEHAVE model is extensively documented and comes with a user manual [30]; thus, in the present study we describe only the overall principles and the changes we made.

The colony module is cohort-based and uses difference equations to simulate the colony dynamics. The foraging module is agent-based and simulates the foragers’ behavior and nectar and pollen collection. The landscape is flexible and describes spatiotemporal nectar and pollen availability, with foraging activity dependent on both landscape structure and weather patterns (for landscape settings in the present study, see sections Control settings and Landscape mitigation scenario). The BEEHAVE model is implemented in NetLogo [35] and is extensively documented; for a full model description, see Becher et al. [30] and the BEEHAVE website [36].

In the present study, “control” denotes scenarios without pesticide exposure; all pesticide scenarios were paired with control scenarios that had identical settings apart from the pesticide effects unless otherwise stated.

Changes to BEEHAVE

All scenarios assessed in the present study were based on the scenario used for pesticide exposure (JPEbecherSA7_modB-hive-Pesticide.nlogo) in the supplementary materials of Becher et al. [30]. In that scenario, the landscape consists of a single forage patch with constant nectar and pollen flow located 1000 m from the colony. The weather in that scenario is from Rothamsted, Harpenden, United Kingdom; the year is 2009 and mortality is implemented as a multiple of the control per foraging trip (MORTALITY_FACTOR) and only affects active foragers. The changes to this scenario and BEEHAVE are outlined below. Most changes to the Becher et al. [30] scenarios could be carried out with changes to parameter values and input files. However, the following changes to the code were necessary: Two new parameters were introduced to allow BEEHAVE to run control setting for some years before exposure commenced (i.e., a spin-up phase) and again for some years after exposure to study recovery. In addition, code was added to allow the removal of a fixed daily proportion of foragers and in-hive bees during exposure periods with the introduction of new parameters to set this proportion. Finally, code was added so MORTALITY_FACTOR could be inputted via the landscape input file (INPUT_FILE), where an extra column was added to facilitate this. A full description of all changes to the BEEHAVE version published in Becher et al. [30] can be found in the Supplemental Data. The altered version of BEEHAVE is also available in the supplementary materials under General Public License (GNU) [37].

Control settings

The purpose of the control scenarios was to explore how colonies in BEEHAVE respond to different settings regarding forage quality. Subsequently, these findings were used to explore how forage quality affected colony resilience to pesticide-induced workers losses.

Weather is important for colony dynamics, as it controls when foraging can take place, so we aimed to standardize weather to avoid results being driven by weather conditions in a particular year. Becher et al. [30] used weather from Rothamsted in 2009, whereas we downloaded the average weather for Rothamsted (1981–2010) from the Met Office online database [38] (monthly averages of daily maximum temperature [°C], sunshine [hours]), which we interpolated linearly to obtain daily values.

We used the same rules as Becher et al. [30] to calculate foraging hours per day (i.e., sunshine hours per day when the daily maximum temperature was above 15 °C). When we inspected the graphs, however, it was clear that average weather is not the same as standard weather; thus the threshold of 15 °C led to a very abrupt onset of foraging (Figure 1), and colonies

![Figure 1. Weather and forage availability used in simulations. Rothamsted mean (primary y-axis): foraging hours per day calculated using mean temperature and sunshine hours from 30 yr of data (1981–2010), with foraging threshold of 15 °C max (total hours foraging: 936). HoPoMo Forage (secondary y-axis): forage availability as proportion of maximum and SHIFT_G set to –30, which shifts curve to coincide with forage season. Weatherwax (primary y-axis): foraging hours smoothened to reflect clement days that would occur in most years (total hours foraging: 963).](image-url)
struggled to survive. In reality, in most years colonies will experience a few good early foraging days with increasing foraging as the weather warms up, and there are also a few good days toward the end of the season. We therefore devised a window function (Weatherwax) to smooth the weather. An Excel spreadsheet with the weather data as downloaded, the interpolation, and Weatherwax function can be found in the Supplemental Data.

Weatherwax function. We used a modified Vorbis window function [39] to estimate the number of hours that bees forage throughout a season. To maintain consistency with the original nomenclature, we define a Weatherwax Foraging Function as

$$F_t = 0 \text{ for } day < D_{\text{start}}$$

(1)

$$F_t = F_{\text{max}} \sin \left( \frac{\pi}{2} \sin \left( \pi \frac{\text{day} - D_{\text{start}}}{D_{\text{period}}} \right) \right) \text{ for } day > D_{\text{start}}$$

and

$$day < D_{\text{start}} + D_{\text{period}}$$

(2)

$$F_t = 0 \text{ for } day > D_{\text{start}} + D_{\text{period}}$$

(3)

where $F_t$ is the number of hours that bees forage in a day; $F_{\text{max}}$ is the maximum number of hours that bees can forage; $D_{\text{start}}$ is the day number that bees start foraging; $D_{\text{period}}$ is total number of days that bees forage; and $D_{\text{day}}$ is the day number.

The Weatherwax Function was smooth and symmetric at approximately the middle of the foraging period calculated according to Becher et al. [30] and fitted it sufficiently well (Figure 1).

Forage availability. Becher et al. set food flow to be constant across the year, but we used the more realistic HoMoPo setting for seasonal food flow [30,40]. The first day of foraging was calibrated to achieve overlap between foraging season and forage availability (Figure 1) (SHIFT_G, which controls the HoMoPo food flow, was set to −30). The single forage patch in the landscape (2 patches for the landscape mitigation scenario; see section Landscape mitigation scenario) did not represent a particular crop or habitat but was a representation of general forage availability and distance in the landscape with the implicit assumptions that during exposure all foragers would forage exclusively on the exposed crop.

Scenarios

Control boundaries for survival scenarios. In BEEHAVE, the colony requires a minimum quantity of energy (nectar) and protein (pollen) to survive. However, the actual quantities needed depend on the sugar concentration of the nectar, the distance between colony and forage, handling time, and total hours available to forage in the year. In the present study, we use the term “boundaries for survival” to denote the combinations of parameter values for nectar sugar concentration, amounts of nectar and pollen, handling time for pollen and nectar, and distance between colony and forage patch for which colonies can survive in BEEHAVE.

The changes to the weather scenario and the introduction of seasonal food flow resulted in different boundaries for colony survival compared with Becher et al. [30]. In the present study, we aimed to establish the foraging quantity required for colony survival in the absence of pesticide exposure by running a series of preliminary scenarios to explore the impact and interaction between distance between forage and hive (hereafter called distance), sugar concentration in nectar, handling time, and quantity of pollen and nectar. These boundaries for survival have implications for lower-tier calculations of exposure in regulatory risk assessments [29] because the lower the nectar sugar concentration that foragers exploit, the more nectar they will need to meet the colony’s energetic needs; hence, all else being equal, they will potentially be exposed to a higher pesticide dose per unit energy.

To compare the scenarios, we calculated the energetic efficiency (the ratio of energy gained from foraging relative to the energy spent foraging) at the beginning of the day according to the equations from Becher et al. [30]:

$$\text{Energetic Efficiency} = \frac{(\text{nectarConcFlowerPatch} \times \text{CROPVOLUME} \times \text{ENERGY}_\text{SUCROSE}) - \text{flightCostsNectar}}{\text{flightCostNectar}}$$

(4)

where nectarConcFlowerPatch is the sucrose concentration of nectar (mol sucrose/L), CROPVOLUME is the crop size of foragers (50 µg), and ENERGY_SUCROSE is 0.00582 kJ/ micromol. Then

$$\text{flightCostsNectar} = (2 \times \text{distanceToColony} \times \text{FLIGHTCOSTS}_\text{PER-m}) + (\text{FLIGHTCOSTS}_\text{PER-m} \times \text{handlingTimeNectar} \times \text{FLIGHT\_VELOCITY} \times \text{energyFactor\_onFlower})$$

(5)

where distanceToColony is distance between colony and forage patch (m), FLIGHTCOSTS_PER_m is 0.000006 kJ/m, handlingTimeNectar is the time spent on forage patch (s), FLIGHT\_VELOCITY is 6.5 m/s, and energyFactor_onFlower is 0.2 (proportion; no unit), assuming the forager spends 20% of the energy on patch compared with the flight to and from patch.

In BEEHAVE, the handling time increases as nectar and pollen is depleted by the foragers during each day, so the calculated energetic efficiency was the daily maximum (pollen and nectar are replenished once per day in BEEHAVE).

As a result of these preliminary scenarios, the following control settings were used in the remaining scenarios: The forage availability followed HoPoMo with SHIFT_G−30, which adjusts the timing of the pollen and nectar flow (see Becher et al. [30] for details). Pollen peak availability was set to 1 kg/d, nectar peak availability to 10 L/d, and sugar concentration in nectar to 2 mol sucrose/L. The Weatherwax settings were as follows: foraging started on day 55, total number of days foraging was 250, and peak foraging rate was 6.4 h/d. This led to a yearly total of 963.5 h of foraging. In reality, most foragers are found within 1 km of the hive if forage is plentiful, but they will go much further if no forage is closer [24]. Colonies have a high probability of finding forage within a 2000 m distance, but little probability at more than 3200 m [24]. So we chose to run all scenarios with distance between hive and forage at 50 m, 500 m, and 1000 m, to cover situations in which a flowering crop is likely to provide a large proportion of the forage collected by the colony.

Fixed worker mortality scenario. This scenario series matched colony assessment studies in which the total number of workers is assessed. It was created to predict the colony’s resilience to loss for set (fixed) proportions of workers at different times of the year. This scenario was used to explore protection goals for colony size reduction. Mortality was imposed as set proportions of all workers (i.e., in-hive bees and foragers) irrespective of whether they were actively foraging and did not take the mechanism of exposure into account. Fixed worker mortality was initially implemented with a single day duration (10%, 25%, 50%, or 75% of workers removed), which took place once a year, at different times: 1 April, 1 May, 1 June, 1 July, or 1 August (flowering crops are rare outside of this
period) in a fully factorial design. The mortality was not related to background mortality, nor was it intended to represent realistic pesticide mortalities, but rather to explore the interaction between timing and magnitude of worker losses.

To explore the importance of duration of exposure, we ran an additional scenario series where total workers losses of 20% and 50% were distributed over 1 d, 3 d, 7 d, 14 d, and 30 d starting on 1 June (which preliminary runs had shown was a sensitive period). This daily mortality was calculated by dividing the total percentage worker lost by duration.

**Forager mortality per trip scenario.** In this scenario, mortality was implemented in the same way as in Becher et al. [30]—that is, as a multiple of control mortality per foraging trip—and was used for comparison with the trigger values for forager losses in the EFSA guidance document [29]. Mortality was implemented at the foraging patch, so it only affected active foragers visiting the patch; thus at each visit the control mortality was multiplied by a set multiplication factor. This type of mortality can represent acute mortality or disorientation where the forager fails to return to the hive; in the latter case the forager would in effect be dead to the colony. This type of mortality implementation matches the data that can be gathered from RFID studies, where foraging activity and return to hive is measured [34]. In this scenario series, the following factors and effect levels were varied in a fully factorial design: distances between forage patch and hive were 50 m, 500 m, and 1000 m; the patch was exposed for 1 d, 3 d, 7 d, 14 d, 30 d; and the mortality level was set at 2, 3, 5, and 10 multiples of control, with onset of exposure set to start 1 April, 1 May, and so forth, until 1 August.

As this type of mortality happens at patch, the overall mortality was emergent and depended on control mortality, number of visits, and proportion of active foragers. The relationship between forager death per trip as a multiple of control and total forager loss is not straightforward to estimate because the total loss is dependent on both the number of active foragers and foraging activity; that is, the more flights per day an individual bee makes, the higher its mortality risk per day, and the greater the total number of active foragers, the larger the total forager loss. Similarly, even a high mortality risk leads to no mortality of foragers outside the foraging season, because they are not active and therefore not exposed.

**Landscape mitigation scenario.** The mitigating effect of alternative unexposed forage was explored by adding an extra foraging patch to the landscape with availability of nectar and pollen set at 10% of control peak abundance (equivalent to a seasonal peak of 1 L nectar and 100 g pollen per day) and followed the same seasonal variation as the main forage patch. The alternative patch was unexposed and only present during the exposure period, which lasted for 30 d starting 1 June. The alternative patch was placed at 50 m, 500 m, and 1000 m from the hive, and the exposed patch was placed 1000 m from the hive. The mortality in this scenario series was implemented as in the forager mortality per trip scenario, but with a MORTALITY_FACTOR of 5 to enforce clear colony level effects.

**Data analysis**

All scenarios were run with 10 replicates. In BEEHAVE, the colony overwintering survival is determined on 31 December, so the data analysis mainly focused on colony size at 31 December after 3 yr of consecutive exposure events (see Figure 4 for an illustration) to account for year on year use of a given pesticide while taking into account the fact that queens are unlikely to last longer than 3 yr [23]. To address protection goal setting, a safe level of forager losses was defined as no risk of colony loss and a maximum of 10% reduction in overwintering colony size after 3 yr of consecutive exposure events.

**RESULTS**

**Control boundaries scenario**

The peak and minimum size of the colony decreased with increasing distance to forage (Figure 2A). The timing of peak colony size also varied with distance from mid-summer at 50 m to late summer at 1000 m. Similarly, the minimum colony size was in early spring at 50 m but in mid-spring at 1000 m (Figure 2A). The difference in timing of peak size was more marked for honey stores, which was at peak for a several months at the 50 m distance but only for a few weeks at 1000 m (Figure 2B). Thus, the colony was at the threshold of starvation at 1000 m; if the sugar concentration was lowered or distance to forage or handling time were increased, the colony went into decline (Supplemental Data, Figures S1 and S2). The colony only survived within certain boundaries of energetic efficiency (Equation 4). With the weather scenario used, a minimum energetic efficiency of 25 was necessary for the colony to survive (Figure 3). Thus, for example, increasing sugar concentration in nectar could to some extent compensate for greater distance between colony and forage or for longer handling times. In BEEHAVE, the control (i.e., without pesticides) mortality of foragers depends on both distance flown and time spent foraging. Therefore, energetic efficiency alone could not fully explain colony survival, because the forager mortality per energy unit brought back to the hive was affected by handling time, distance, and nectar sugar concentrations. For instance, with low nectar sugar concentrations, the foragers would have to fly more trips to bring back the same amount of energy and therefore flew longer and consequently suffered higher mortality. In contrast, increasing the abundance of nectar and pollen beyond a saturation threshold only had limited potential to compensate for poor energetic efficiency or high forage-related mortality (as a result of low sugar concentration or high distance or handling time; Supplemental Data, Figure S2). This was to a certain degree an artifact of BEEHAVE’s default threshold for how much honey the bees

![Figure 2. Seasonal colony dynamics for control hives at different distances from the colony to the forage patch: 50 m (purple line), 500 m (blue line), and 1000 m (green line). The x-axis represents the day in year. (A) Colony size as number of workers. (B) Size of honey stores (in kg of honey).](image)
Fixed worker mortality scenario

The time to recovery after a forager loss depended on forage quality (in the present study, implemented as distance). For instance, at 50% adult bee mortality, the colony recovered before winter at 50 m from forage but failed to fully recover at 1000 m from forage (Figure 4). The higher the proportion of workers removed on a single day, the larger the impact on the overwintering size of the colony (Figure 5). Mortality interacted with distance; so at 50 m there was hardly any impact on overwintering colony size of even high worker mortalities, whereas at 1000 m distance the overwintering size decreased rapidly when worker mortality exceeded 20% to 30% (Figure 5). The timing of the effect was also important, with effects late in the season having larger impact on overwintering size than those early in the season (Figure 5). The colony was more sensitive to worker loss in August because colony size peaked then (resulting in a larger total number of workers being removed), egg laying was already declining, and there was less time and forage to exploit for the colony to recover before overwintering. It was the total size of the adult worker loss that mattered for overwintering size of the colony, whereas the number of days over which the mortality was distributed only had a negligible effect (Figure 6).

Forager mortality per trip scenario

There was no impact of this type of mortality outside the foraging season, as there was no exposure (Supplemental Data, Figure S3). During the foraging season, colony size was reduced with increasing mortality factor and increasing duration of exposure (Figure 7), which both led to larger total loss of workers. Again, there was interaction with distance, so the colony resilience decreased with increasing distance. Mortality and duration also interacted with timing of exposure (Figure 7), but the worst-case timing of exposure (April and June) was different from that in the fixed worker mortality scenario. The reason for this was the way in which mortality was implemented in the 2 scenarios; in the forager mortality per trip scenario, only foragers were affected, which meant that there was more scope for compensation by lowering the age of first foraging for in-hive bees than in the fixed worker mortality scenario, in which both in-hive bees and foragers were equally affected. A safe level of forager losses was defined as no risk of colony loss and a maximum of 10% reduction of overwintering colony size after 3 yr of consecutive exposure events. At 1000 m distance from hive to field, this resulted in the following trigger values for forager losses: 3 times control mortality per flight for 14 d, 4 times for 7 d, and double for 30 d (Table 1); but at 50 m, the thresholds were much higher (Figure 7).

Landscape mitigation scenario

Adding a small amount of alternative forage to the landscape mitigated the effects of exposure to pesticides for settings that would otherwise lead to colony decline and loss (Figure 8). The mitigating effect was larger if the alternative unexposed forage was closer to the colony than the exposed forage. The effects of the landscape mitigation stemmed partly from the presence of additional resource but primarily from fewer bees being exposed. Thus, when the alternative patch was more energetically efficient, because of shorter distance, it was favored by the foragers. Moreover, when the mortality at the exposed patch was high, fewer foragers were recruited to the exposed patch.

DISCUSSION

Our simulations with BEEHAVE indicated that forage quality plays a critical role in the colony’s resilience to stressors. From the scenarios to explore the boundaries for control colony survival, it appeared that both energetic efficiency (i.e., the energy gained relative to the energy spent acquiring it) and the background foraging mortality per energy unit acquired affected colony resilience. The importance of forage was also reflected in the pesticide scenarios, which in all cases showed that colonies at edge of field had much higher resilience to worker losses than colonies further from the field (when the field was the only source of forage), where energetic efficiency was lower and the background mortality per energy unit acquired was higher. Indeed, colonies adjacent to an exposed field with substantial forager losses fared better than those far from an untreated field. That bountiful and accessible forage close to the hive is important for honeybees is well known and a fact beekeepers exploit by placing hives next to flowering crops or bee-attractive flowers while ensuring colony survival by feeding them sugar syrup and fondant at times of nectar and pollen dearth [41]. Similarly, impoverished forage and habitat loss have been implicated in the decline of several bee species [6,23,41,42], and starvation frequently contributes to honeybee colony losses [43,44].

Because colony survival typically depends on overwintering size and effects may accumulate year on year [30,45], in the present study we propose to set the overall protection goal in simulations as no colony losses and a maximum of 10% reduction in overwintering colony size after 3 yr of consecutive exposure events. The fixed worker mortality scenario, which matches colony assessments in field studies, showed that when the colony was placed at the edge of the field, it made full recovery even after losing 50% of workers. Although losses of this magnitude may appear high, they are similar to worker losses as a result of swarming, which may happen several times a year in thriving colonies [23]. Similarly, beekeepers employ several techniques to reduce swarming and also make new nucleus colonies by splitting to increase numbers.
demonstrating that thriving colonies have substantial compensation mechanisms for reduction in size [41]. In our simulations at 1000 m distance, however, the colony was less resilient and the threshold for full recovery within a season was approximately 20% to 30% worker losses. Although our results are specific to the forage availability and weather pattern used in the scenarios, which were calibrated to stress the colony, the losses that BEEHAVE predicted a colony could sustain are much more in line with knowledge of bee biology (e.g., swarming), losses associated with movement of hives, and beekeeping practices [23,41] than the protection goal of 7% reported in EFSA bee guidance document [29]. Thus, EFSA reported protection goal of 7% of colony size is overly conservative given the large natural fluctuations found in colony size over the season and between years [16,17].

The trigger values that EFSA calculated for forager losses using the Khoury et al. model [28] were set as a multiple of control [29]; however, it is not clear whether they mean per trip or per day, and, as our analysis showed, these 2 are not necessarily the same. Using the same protection goals we described (i.e., 10% reduction in overwinter size after 3 yr with consecutive exposure events), our results indicated that forager losses should not be greater than double the control mortality per trip for 30 d, whereas EFSA [29] states that double control mortality is only acceptable for 3 d [29]. Similarly, we showed that 3 times control mortality per flight only caused negligible effects if it lasted for 14 d, whereas EFSA states that the limit for negligible effects for 3 times the control mortality is 2 d. Similarly, our simulations showed that if the effects were short lived (up to 3 d), even increasing mortality to 10 times the control mortality per trip had no discernable effects on overwintering colony size. Implementing similar mortalities (i.e., 1.5–3 times control) and durations (i.e., 2–6 d) in BEEHAVE had hardly any discernable effects on colony size even at the time of exposure, and none on overwintering size. To set their limits for forager losses, EFSA used the Khoury honeybee model [28], which excludes honey stores, seasonality of forage and colony dynamics, energy budgets, and many of the regulation mechanisms employed by bees to deal with stressors and changing forage availability [25].

This is not meant as a criticism of Khoury et al. [28], as the authors clearly state that “the aim of this model is simply to

Figure 4. Example of colony dynamics over 3 yr with a yearly event where 50% of workers were removed on 1 June (fixed worker mortality scenario). (A) Colony size; the red vertical line shows the colony size on 31 December after 3 yr of consecutive exposure events, which is used as an endpoint in other graphs. (B) Honey stores. The x-axis represents time, and dotted lines indicate exposure events. Distance = distance between colony and forage patch (m).
provide a basic theoretical understanding of colony dynamics in an idealized state.” However, it does highlight the importance of including the factors that are driving a biological system and understanding the limitations and strengths of different modeling approaches [46,47]. Beekeepers employ a number of management techniques to counteract stressors; for example, during years when forage or weather is poor, they either reduce the honey harvest or feed the colonies or face the consequences through colony loss [41]. We recommend that the control scenario for pesticide risk assessment should be based on a stable colony (i.e., it should not be based on a declining colony that is doomed to fail), but one that is only just stable, to ensure conservatism. In the present study, we used food stress as means to calibrate BEEHAVE so the control colony was stable but vulnerable. In contrast to pesticide-induced stress, parasites and diseases reproduce and multiply; therefore, even with unlimited forage and good conditions, the colony may not be able to compensate for the bee losses [30]. Beekeepers routinely control for Varroa mites and diseases to ensure colony survival [41]. Therefore, vulnerability could also be calibrated in other ways, such as by adding other stressors such as Varroa, but then the level of foraging stress would have to be reduced to ensure control colony survival. Such calibrations should be carried out for representative regions, as weather, landscape structure, and beekeeping practices will all have a substantial impact on colony resilience. The European Food Safety Authority states that one of the main obstacles in using BEEHAVE for setting protection goals is the lack of a pesticide module [31]. In the present study, we demonstrated how some fairly simple changes can overcome the current limitations of BEEHAVE as published, but it also became clear that because the weather, landscape structure, and forage quality have such large impacts on colony dynamics, work is needed to develop realistic worst-case scenarios. Ideally, a group consisting of experts on diseases, parasites, forage, beekeeping practices, agronomy, landscape ecology, and ecotoxicology would define the control scenarios for what constitutes realistic worst-case scenarios in terms of landscape structure, beekeeping practices, and weather in the different regions.

In our scenarios, the colony needed very good energetic efficiency to survive, and it seemed that compared with typical distances flown by foragers and sugar concentrations in nectar, this was on the hungry side [19,20,23]. The reason for the discrepancy between our simulations and natural foraging is probably that we used the same sugar concentration and distance to forage all year, which meant that the colony missed out on some good foraging opportunities that are known to be important for bees, especially if they occur early in the year [41]. However, as long as the food availability for the control scenarios are calibrated so that the colony is on the brink of

Figure 5. Overwintering size of the colony when different proportions of workers were lost (fixed worker mortality scenario). The y-axis represents overwintering colony size (number of workers on 31 December) after 3 yr of consecutive exposure events. The x-axis represents the proportion of foragers and in-hive bees removed. The dotted line shows the European Food Safety Authority’s protection goal of 7% reduction of colony size [29]. Legend colors indicate the month of exposure (first day of month). Distance = distance between colony and forage patch (m).

Figure 6. Overwintering size on colony when a given cumulative fixed mortality was distributed over different durations. Exposure started 1 June. The y-axis represents overwintering colony size (workers on 31 December) after 1 yr with 1 exposure event, and the x-axis represents total mortality (proportion of workers removed; no unit). The legend refers to the number of days over which the total mortality was distributed.
starvation, then the impact of any stressors will be conservative. The sugar concentrations that can sustain a colony depend on the energetic efficiency and background mortality per energy unit acquired, but also on the length of the foraging season and weather. For instance, in the Becher et al. study [30], in which the nectar flow was constant and the foraging season longer, the colony could survive on lower nectar sugar concentrations than in the simulations presented in the present study, where the foraging was more restricted. However, the sugar concentration of 15% used by EFSA in their calculation of shortcut values used for exposure and risk assessment is probably on the low side for colony survival, unless the handling time is extremely

Figure 7. Colony overwintering size at different exposure durations and mortality levels for foraging trips (forager mortality per trip scenario). The y-axis represents colony overwintering size (workers 31 December) after 3 yr of consecutive exposure events. The x-axis represents mortality factor (i.e. a multiplication factor by which the control background for each trip is multiplied). Legend colors indicate exposure start (first day of month). Distance = distance between colony and forage patch (m); Exposure duration = the number of days that the exposure lasted.

Table 1. Colony size (workers on 31 December after 3 yr of consecutive exposure events)a

| Treatment dayb | Treatment periodc | 1   | 2   | 3   | 4   | 5   | 10  |
|----------------|-------------------|-----|-----|-----|-----|-----|-----|
| 1 April        | 1                 | 19845 | 20103 | 19636 | 19680 | 19671 | 19713 |
|                | 3                 | 19845 | 20787 | 19317 | 19758 | 19453 | 19146 |
|                | 7                 | 19845 | 19882 | 19752 | 19865 | 19346 | 14230 |
|                | 14                | 19845 | 19860 | 19183 | 18731 | 15886 | 3994 |
|                | 30                | 19845 | 20124 | 17853 | 14792 | 8507  | 1215 |
| 1 May          | 1                 | 19730 | 19713 | 19601 | 19787 | 19737 | 19961 |
|                | 3                 | 19730 | 19849 | 19844 | 19643 | 19896 | 19269 |
|                | 7                 | 19730 | 19294 | 19410 | 19831 | 18930 | 17580 |
|                | 14                | 19730 | 19508 | 19597 | 18388 | 18799 | 16376 |
|                | 30                | 19730 | 19269 | 17988 | 11723 | 9330  | 0    |
| 1 June         | 1                 | 19977 | 19649 | 19994 | 19914 | 19209 | 19966 |
|                | 3                 | 19977 | 19587 | 19784 | 19544 | 19832 | 19525 |
|                | 7                 | 19977 | 19423 | 19564 | 19790 | 18926 | 15785 |
|                | 14                | 19977 | 19852 | 18985 | 17045 | 16226 | 5726 |
|                | 30                | 19977 | 18584 | 11786 | 2511  | 0     | 0    |
| 1 July         | 1                 | 19651 | 19960 | 19541 | 19907 | 19695 | 19860 |
|                | 3                 | 19651 | 20322 | 19903 | 19616 | 20004 | 19733 |
|                | 7                 | 19651 | 19636 | 19541 | 18611 | 19187 | 18422 |
|                | 14                | 19651 | 19816 | 18583 | 17631 | 16521 | 13641 |
|                | 30                | 19651 | 18297 | 14445 | 6735  | 0     | 0    |

aThe forage was placed 1000 m from the colony. Bold text Indicates more than 10% reduction in overwintering colony size.
bStart of exposure.
cDuration of exposure (in days).
dFactor by which control mortality per flight was multiplied (1: control).
short. Thus, for a handling time of 1200 s (which is typical according to Winston [23]), the energetic efficiency for 15% sugar in nectar is only 12 even at edge of field, and thus half of the threshold for colony survival of 25, which we identified in our scenarios. As a consequence, the overall percentile of the exposure implicit in EFSA shortcut values is likely to be far higher than the 90th percentile exposure value EFSA aimed for.

Although neonicotinoid residues generally are detected in less than 5% of samples [48], there has been much debate over whether the use of neonicotinoids as seed treatments have caused widespread colony failures through exposure via pollen and nectar [12,13]. Several researchers have found increased forager mortality in laboratory and semIELD studies [12], but the realism of exposure concentrations has been questioned [14] and effects on colony dynamics have not been detected in field studies with naturally foraging honeybees [16,17]. Moreover, it appears that the increase in overwintering colony losses plateaued or was reversed before the neonicotinoid moratorium in Europe came into place [2], and it has also slowed in the United States, where neonicotinoids are still used [9]. Our results indicated that if mortalities exceed the recovery potential of the colony, the effects build up and the resultant reduced colony size would be clearly visible in colony assessments and honey yield (e.g., Figures 4 and 6). Thus, the apparent concern that effects can build up undetected until the colony suddenly collapses seems unfounded, especially during targeted regulatory studies, in which the colonies are frequently monitored and for extended periods post exposure [32].

Our mitigation scenario indicated that a small amount of alternative forage can reduce the exposure to pesticides on crops if it is of a better quality than the crop, in the present study simulated by a shorter distance. Honeybees choose the most energetically efficient forage and if there is more attractive forage available, sufficient pollination of, for example, apples (which only have 25% sugar in the nectar) can be challenging [41]. Together with our findings from the control boundaries for survival scenario and the pesticide scenarios, this points to the critical role of increasing the abundance of good-quality forage in agricultural landscapes to increase the resilience of honeybees toward the stressors in general. Long-term changes to farming practices have led to far fewer flowers in the agricultural landscapes; for example, over 90% of the flower-rich meadows have been lost in the United Kingdom since the 1950s [49], clover in pastures has become much less prevalent, and more effective weed control has resulted in less and less continuous forage for pollinators [50]. However, the lack of flowering weeds within fields also means that fields become less attractive to pollinators, thus reducing exposure to pesticides outside the flowering period of the crop itself [51]. Beekeepers often feed colonies to increase overwintering survival [41]. However, several studies have shown that increased availability of forage on farms (e.g., improved field margin management such as sowing of flower margins and strips) also benefits other wild bee species [52], again highlighting the importance of improving forage availability for pollinators in agricultural landscapes.

We recommend setting protection goals using stressed honeybee colonies at the threshold of decline to ensure conservatism. The results showed that total cumulative loss of workers drives the colony response rather than the number of days the loss is distributed over. We conclude that for such suboptimal conditions, a total 20% reduction in colony size is a realistic worst-case protection goal.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3504.

Acknowledgment—We thank M. Becher for help with changes to the code and valuable discussions about the results and mechanisms. We thank V. Grimm for constructive comments on the manuscript.

Disclaimer—The authors are employed by Syngenta Ltd.
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Data availability—Data are available on request from the corresponding author at permille.thorbek@syngenta.com.

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