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COLONY IMPACT OF PESTICIDE INDUCED SUBLETHAL EFFECTS ON HONEYBEE WORKERS: A SIMULATION STUDY USING BEEHAVE

Running title: Simulating sublethal effects on bees with BEEHAVE

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Abstract: Research on neonicotinoids and honeybees have changed focus from direct mortality to sublethal effects. In the present study, we use a published honeybee model, BEEHAVE, to compare induced colony level impact of pesticides including direct mortality, poor brood care, disorientation, increased handling time in oilseed rape and sunflower crops. Actual effects on individual bees will depend on exposure concentrations, but in the present study large effects were enforced. In oilseed rape, poor brood care had the largest colony impact as it created a bottleneck for spring build-up of the workforce, and colony impact for all effect types peaked a month after exposure ceased. In sunflower, the later exposure changed the response so colony impact peaked during exposure and the bottleneck was honey store build-up. In all scenarios, good forage mitigated effects substantially. We conclude field studies should continue at least one month after exposure to ensure detection of ecologically relevant sublethal effects. The results indicated that even if a sublethal effect is difficult to detect in the field, subsequent ecologically relevant colony level impacts would be clear if studies are continued for one month after exposure. Guidance for regulatory studies recommends extended observation periods and published field studies already use extended observation periods, so we conclude current methods are adequate for detecting ecologically relevant sublethal effects. While published laboratory and semi-field studies conducted under controlled exposure conditions suggest sub-lethal effects may occur, published field studies with neonicotinoid seed treatments, naturally foraging bees and extended observation periods do not report colony level effects, suggesting that in these studies no ecologically relevant sublethal effects occurred.

Keywords: Ecological risk assessment, Environmental modeling, Population modeling, Population-level effects, Ecological modeling, Honeybee

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INTRODUCTION

Overwintering losses of European honeybee (*Apis melifera* L.) colonies has been intensively studied and reported over the last decades [1-3]. While honeybees are exposed to multiple stressors e.g. the varroa mite (*Varroa destructor*) and the viruses they vector, lack of forage, Nosema and changes to beekeeping practices and profitability [1, 4-8], the debate has centred on the impact of pesticides, in particular neonicotinoids [9, 10]. The research on neonicotinoids and honeybees have changed focus over the years; at first the focus was on mortality of honeybees from different routes of exposure e.g. dust [11, 12], guttation [13] and consumption of nectar and pollen [14]. However, neonicotinoid exposure concentrations in the nectar and pollen from plants grown from coated seeds are generally too low to cause widespread forager mortalities [10, 15, 16]. Nonetheless, several studies have demonstrated that under controlled dosing experimental conditions neonicotinoids may cause sublethal behavioural effects at exposure concentrations lower than those which would cause mortality [14, 17]. Behavioural effects such as reduced mobility, learning [18-20], orientation [21, 22], slower return to hive, [21], longer duration of foraging flights [21, 23] have been recorded. Other sublethal effects appear to be physiological such as effects to the hypopharyngeal gland which may reduce brood food production [24]. Most of these tests have been performed in the laboratory using methods such as the proboscis extension reflex or histology which are challenging to translate into quantifiable effects on the individual's performance of e.g. foraging or brood care under field conditions.

There appears to be a concern that because sublethal effects can be identified under highly controlled laboratory conditions, but are difficult to quantify in colonies under more variable field conditions, such effects may build up unnoticed until the colony seemingly inexplicably collapses [14, 22]. While impact on forager activity can be detected by, for instance, using radio frequency identification (RFID) studies [23, 25, 26], any impact on the ability of nurse bees to care for the brood, is much harder to detect within a colony. Moreover, it is not straightforward to predict what the impact of sublethal effects on individual workers would be on colony size or survival [17]. Honeybees are social insects that employ many sophisticated mechanisms to cope with changing forage flows and stressors (e.g. adjusting age of first foraging, cannibalism of brood in times of limited resources or prioritisation of pollen foraging during shortages). Therefore, colony level effects are not necessarily proportional to the individual-level effects and effects are not necessarily additive [27]. For instance, if cognitive impairment affects orientation, the foragers may fly longer to collect the same amount of nectar thus reducing the return in energetic terms. This in turn could reduce the colony's honey stores for winter, potentially impacting the colony long after exposure, which would make the design of field studies to detect such prolonged effects challenging.

Therefore, a tool is needed that can predict the colony level impact of sublethal effects on workers. Ecological models are an excellent tool for extrapolating from individual-level effects to population level, or in the case of honeybees to colony level [27, 28]. In order to do this the model should be able to simulate both the sublethal impact on foraging and brood care as well mechanistically represent the colony dynamics and the feedback mechanisms which allow honeybees to respond to changing forage levels and stressors [27]. BEEHAVE is such a model which realistically combines a colony model with both a foraging model and a landscape model [29], and it has been recommended by the European Food Safety Agency as a basis for further development of honeybee modelling in pesticide risk assessment [30].

In the present study we use BEEHAVE to study: (i) what impact sublethal effects have on the colony compared with lethal effects, (ii) when symptoms would occur relative to exposure, (iii) whether mitigation can alleviate effects. Rumkee et al. [31] use BEEHAVE to simulate effects on queen egg laying rate and mortality of different life stages and found the colony was most sensitive to loss of workers, so here we focus on sublethal effects on workers. We simulated three types of sublethal effects and for comparison also a lethal effect: impaired orientation (by doubling distance to forage during exposure), ability to handle flowers (by doubling handling time per foraging trip), effects on brood care (by halving the brood to in-hive bee ratio) and lethal effects (by doubling the mortality per foraging trip for foragers relative to the control mortality per trip). Actual effects on individual bees would depend on the exposure concentrations [15], but here we chose to simulate an effect size that was large enough to cause colony level impact because the purpose was to compare the colony response to different types of effects. Understanding these colony responses will help facilitate improvement of honeybee colony pesticide effect studies (or other stressors) as well as the assessment of data obtained from such studies.

METHODS

BEEHAVE was used for all simulations. BEEHAVE is a honeybee model that integrates processes inside the hive with landscape dynamics via a forager module and it was developed by Becher et al. [29]. BEEHAVE has been developed to study the impact of multiple stressors on honeybee colonies and is based on a review of existing bee models [27]. The colony module is cohort-based and uses difference equations to simulate the colony dynamics. The foraging module simulates the foragers' behaviour and nectar and pollen collection and is agent-based. The landscape is flexible and describes spatio-temporal nectar and pollen availably (for details on the simplified landscape used here, see the scenario descriptions). Foraging activity depends both on landscape structure and weather patterns. BEEHAVE is extensively documented and tested and comes with a manual (for full model description see Becher et al. 2014 and (www.beehave-model.net). BEEHAVE has also been thoroughly reviewed for the suitability to pesticide risk assessment see [30], so here we only describe changes made to BEEHAVE and the scenarios used.

All scenarios were based on the settings used in [32], which again were based on the ones used for pesticide exposure (JPEbecherSA7_modBhave-Pesticide.nlogo) in supplementary materials of [29]. The [32] scenarios used standardised weather based on temperature averages for Harpenden, UK, 1981-2010 with seasonal food flow [32]. The settings for the control forage were as follows: Sugar concentration in nectar (2 mol sucrose/L), handling time (1200s for nectar and 600s for pollen), peak flow of nectar (10L/ day) and pollen (1kg/day); pollen and nectar were replenished daily.

The landscape used in the present study had one main forage patch with nectar and pollen availability as for the control patch, which represented general forage availability in the landscape and so it was assumed that during exposure period all that forage was exposed. In different scenarios, the main forage was placed at 50m, 500m or 1000m representing decreasing forage quality. The distance from the colony (hereafter distance) to forage affects both the energetic efficiency of forage (i.e. the energy brought back relative to the energy cost of collecting it) and the mortality per energy unit acquired (see [32] for details). In the Mitigation Scenarios, an additional unexposed patch was present only during exposure period of the main forage patch. With the weather used here, these settings keep colonies placed 1000m from forage at the threshold of starvation while colonies at 50m and 500m distance perform much better [32].

Changes to BEEHAVE

The changes to [32] scenarios and BEEHAVE are summarised in this section; full description of all changes to the BEEHAVE version published in [29] can be found in supplementary materials. The altered version of BEEHAVE used here is available in the supplementary materials under GNU Public license vs 3.

Pesticide scenarios

We developed two exposure scenarios to represent two bee attractive crops namely winter oilseed rape (OSR) and sunflower. For the OSR scenario exposure started 15 April and lasted for 30 days; for the sunflower scenario the exposure started 15 July and lasted for 30 days. In order to create different levels of background stress, the pesticide scenarios were run with three different control distances from colony to field (forage patch) of 50m, 500m and 1000m. The main forage patch represented general forage availability in the landscape and not nectar and pollen availability of the specific crops; thus the assumption was that all forage in the landscape (except mitigation patch if present) was exposed during the bloom of the crop.

The pesticide effects doubled the effect relative to the control, except in the mitigation scenarios where it was tripled to create larger effects. In the exposure period the following effect types were implemented separately:

Direct Mortality Scenarios. As in Becher et al. [29] mortality was implemented as a multiple of control mortality per foraging visit and was implemented at foraging patch. Therefore mortality only affected active foragers visiting the patch, i.e. at each visit the control mortality is multiplied by a pre-set multiplication factor (2 in this scenario).

Disorientation Scenarios. In order to simulate disrupted orientation caused e.g. by learning or cognitive impairment, the distance between hive and forage patch was doubled during the exposure period resulting in distances during exposure of 100m, 1000m and 2000m (i.e. double the controls), thus, simulating that the foragers were struggling to find the way to and from the forage patch. It was assumed that the energetic cost of foraging per flown metre was the same as for control and that the foragers responded to how far they had flown when assessing energetic efficiency.

Handling Time Scenarios. In BEEHAVE the handling time is defined as the time spent at forage patch for one foraging trip and include the time spent handling flowers as well as the time spent flying from flower to flower and it is assumed that the energetic cost is 20% of that spent flying to the patch from the colony [29]. In order to simulate a reduced ability to handle flowers for pollen and nectar gathering and/or ability to choose the best route within the forage patch caused e.g. by learning, cognitive or motor impairment, the handling time per trip was doubled relative to the control (i.e. from 600 to 1200s for pollen and 1200s to 2400s for nectar). The energetic cost per second handling time was kept the same as the control and it was assumed the foragers responded to how long they had spent at patch when assessing energetic efficiency.

Brood Care Scenarios. In order to simulate sublethal effects on nurse bees which would reduce their capacity to care for brood (e.g. via effects on the hypopharyngeal gland, reduced mobility or impaired cognitive function), the ratio of brood to in-hive bees was reduced from 3 (default in [29]) to 1.5, i.e. instead of being able to care for 3 larvae a nurse bee could only care for one and a half larvae.

Landscape Mitigation Scenarios. In order to study the mitigating effect of alternative unexposed forage we developed a scenario series for the Handling Time Scenarios for oilseed rape and for sunflower and for the oilseed rape Brood Care Scenarios, which preliminary runs had shown had relatively large effect on colony dynamics. In the mitigation scenarios individual-level effects were tripled to create larger colony level effects (i.e. handling times for pollen foraging were increased to 1800s and nectar to 3600s and the brood to in-hive bee ratio was decreased to 1:1).

In the landscape mitigation scenarios, an additional unexposed patch was present only during exposure periods. The alternative patch had 10% of the pollen and nectar of the main patch and was placed at 50, 500, or 1000m from the colony to test different levels of attractiveness relative to the main patch (foragers in BEEHAVE prefer the most energetically efficient forage). The alternative patch was only added in conjunction with the 1000m main patch (which was present all season), which preliminary runs had shown to result in greater sensitivity to additional stressors than the shorter distances. In the sunflower handling Time Mitigation scenario an extra series where nectar and pollen availability of the alternative patch was varied in steps from 10 to 50% of the exposed main patch.

Data analysis

All scenarios were run for 3 years to allow testing whether consecutive exposure events would lead to build up of effects [29, 32, 33], while taking into account queens are unlikely to be productive for longer than 3 years [34]. All scenarios were run with 30 replicates and all results refer to the average of those. Numbers of eggs, larvae, pupae, in-hive bees, foragers and drones were recorded and the mass of honey and pollen stores and the total mass of bees and stores were also recorded. All pesticide scenarios were matched with control scenarios, which were identical except for the direct mortality and sublethal effects in the pesticide scenarios. As losses can build up year on year, all figures show the results from the third year.

RESULTS

In the control scenarios, the distance from forage patch to hive (hereafter distance) affected both timing of peak and duration of peak of colony size (number of workers) and honey stores. Thus at 1000m the colony size peaked one month later than at 50m and while the honey stores were at peak size for almost a 100 days at 50m, the peak duration was only about a week at 1000m (colony size: Figure 1, Figure 5; honey store supplementary material Figure S1) indicating that at 1000m the colony was at the threshold of starvation.

Oilseed Rape Scenarios

Effect on Brood Care resulted in the largest colony level impact, Handling Time and Direct Mortality had intermediate colony level impact while Disorientation only resulted in negligible effects on colony dynamics (Figure 1). The effect of Brood Care was mainly driven by high levels of larval mortality resulting from the lack of care by in-hive bees (Figure 2). Direct Mortality and Handling Time both resulted in increased larval mortality, but in those scenarios this was mainly indirectly through effects on the levels of pollen stores within the colony (Supplementary Materials Figure S2).

For all oilseed rape scenarios, the reduction of worker bees (Figure 1) typically was at its maximum about a month after exposure and lasted for some time before the colony began to recover. In general the shorter the distance, the faster the colony recovered (Figure 1, Figure 2). However, for the Brood Care effect at 1000m the colony did not make a full recovery (Figure 1) and therefore the effects built up year-on-year (can be seen as a lower colony size at start of year 3 shown in Figure 1). For Disorientation, Handling Time and Direct Mortality, the weight of the colony (mass of all bees of all stages plus pollen and nectar stores, but excluding hive materials) was reduced during exposure, whilst for the impaired Brood Care the maximum weight reduction occurred about one month after exposure (Figure 3).

Oilseed Rape Landscape Mitigation scenarios

Handling time. To assess whether the availability of alternative forage mitigated the effects of exposure, the handling time of pollen and nectar collection was tripled to create larger effects and then an alternative forage patch with 10% of the pollen and nectar of the main patch was placed at different distances (only during exposure). The results indicated that having these relatively small amounts of alternative forage substantially mitigated the effects, irrespective of whether the alternative forage was placed at the same distance or closer to the colony than the exposed forage (Figure 4). The longer handling time, and to a lesser extent the shorter distance to some of the mitigation scenarios, meant the alternative unexposed forage was more energetically efficient and therefore preferred by the foragers, thereby reducing exposure and hence colony level impact (Figure 4).

Brood Care. Adding alternative unexposed forage did not mitigate the effects of Brood Care much (not shown), because in this implementation unexposed forage did not alter the exposure of in-hive bees and hence the toxic effects on in-hive bees remained the same, and the extra forage was not sufficient to increase resilience because it was only available during exposure.

Sunflower Scenarios

Overall, the magnitude of effects were smaller and the colony recovered faster in the sunflower scenarios than in the oilseed rape scenarios (Table 1). The sunflower scenarios differed from the Oilseed rape scenarios in that the exposure happened late summer and that shaped the colony's response to the different types of toxic effects. Thus, the colony level impacts peaked during exposure and when colonies recovered, they did so faster than in the Oilseed Rape scenarios (Figure 5). The ranking of the effects also differed and in the sunflower scenario reduced Brood Care had hardly any colony level impact (Figure 5). The reason was that the reproductive season was coming to an end and exposure coincided with peak in in-hive bee numbers so they could keep up with the brood nursing. Surprisingly, increasing Handling Time, and to a lesser degree Disorientation, led to colony size increases at 50m and 500m distance (Figure 5). This was caused by the poorer energetic efficiency and full honey stores which led to foragers being less active and therefore suffering less background mortality than in the control. In contrast, at 1000m the honey stores were not yet full, so the foragers were active and both Direct Mortality and Handling Time scenarios failed to recover and there were year on year effects (Figure 5). For the Handling Time, in-hive bees matured earlier to compensate for less nectar being brought in, which led to higher indirect forager mortality (foragers suffer higher mortality than in-hive bees) (See Supplementary Materials Figure S3). For both Handling Time and Direct Mortality at 1000m distance, the colony could not compensate fully because the breeding season was coming to an end so the lost foragers could not be replaced until the year after.

Sunflower Landscape Mitigation scenario

Handling time. Only handling time was tested because impaired Brood Care only had negligible effects in the sunflower scenario (Figure 5). Adding 10% alternative forage to the landscape did not have the same mitigating effect as in the oilseed rape scenario (Figure 6). The main reason was that exposure happened so late in the year and at a time when the rate of build-up of honey stores at 1000m was faster than in the oilseed rape scenario (Figure 1, Figure 5), so the 10% extra forage could not compensate fully (Figure 6). The amount of alternative forage had to be increased to 15-25% of the exposed main forage patch for the mitigating effect to be sufficient for the colony to recover before winter (Figure 7).

For both oilseed rape and sunflower scenarios generally the maximum impact increased with distance as did time to recovery (Table 1).

DISCUSSION

We used BEEHAVE [29] to compare the colony level impact of three sublethal effects with Direct Mortality of foragers. Our simulations indicated that some types of sublethal effects can have similar colony level impact as Direct Mortality. Chronic risk assessments are typically carried out as risk quotients, where the lowest toxicity endpoint is compared to an exposure measure, and the level of risk is determined by the size of the risk quotient rather than by the type of sublethal effects. However, while all of the sublethal effects simulated here had the same magnitude of individual effect level (doubled relative to control and in mitigation scenarios tripled), the colony level impact varied considerably depending on the landscape context and type and timing of the sublethal effects. For instance, the most severe colony level impact was caused by Brood Care impairment, but the timing was critical. Thus in spring, impaired Brood Care led to long lasting effects, but in late summer it had no discernible effects. In contrast, in late summer doubled Handling Time had long lasting colony level effects, whereas in spring the colony recovered faster. The importance of timing of the stressors can be understood when seasonal dynamics of BEEHAVE colonies are taken into account. Thus, in spring (oilseed rape scenarios) when the colony needed to build up the workforce, the bottleneck for the colony was brood rearing and hence effects on in-hive bees were worst-case. In late summer (sunflower scenarios) the colony needed to build up honey stores for the winter so the bottleneck was nectar foraging and effects on foragers were worst-case. In spring, doubling Handling Time also had quite large initial effects (even though the colony made full recovery); however, in spring the main impact was caused by pollen shortage, which created a bottle neck for brood development, whereas in late summer the main impact was nectar shortage. The colony level impact of Disorientation resembled Handling Time, but had smaller effect size because the overall energetic cost was lower than for Handling Time.

Our simulations indicated that good forage increases the colony's resilience to sublethal as well as lethal effects and moreover that bee-attractive alternative forage has the potential to reduce exposure to treated crops. The quality of forage was here changed by increasing distance from colony to forage, where the colony was placed at edge of forage patch (50m distance) the colony was in all cases able to recover before winter. The Mitigation scenarios showed that the effectiveness of mitigation depends on the how attractive the alternative forage is relative to the exposed forage and to a lesser degree on how much alternative forage is available. Thus, for the Handling Time scenarios, the increased handling time meant that the energetic efficiency of the alternative patch was higher and so it was preferred over the exposed patch. However, in late summer the alternative patch could not fulfil the colony's need and therefore for the mitigation to be efficient, the pollen and nectar flow from the alternative patch needed to be higher than in spring. The landscape mitigation was not as efficient if the exposure was not changed, thus, adding an alternative unexposed patch did not mitigate the Brood Care effects (because in our implementation this effect did not depend on forage source). Somewhat in contrast to this, the colony recovered more quickly in the scenarios with good forage quality (50 and 500m distance). This was because the better forage allowed the colony to compensate for early losses and recover, whereas in the mitigation scenario, the extra forage was only available during exposure and so could not help the colony recover after exposure ceased. In a Radio Frequency Identification (RFID) study, Thompson et al. [35] found that the further the colonies were placed from the fields the higher proportion of pollen came from other sources. Thus the worst-case combination used here with long distance between colony and forage, is probably unrealistic as the longer the distance the more likely the foragers would be to use other forage sources.

That nectar and pollen availability as well as clement weather which allows foraging is important for honeybee colonies is well known (e.g. [34]) and pollinator decline has been linked to forage shortages [36]. BEEHAVE has been used to demonstrate that forage quality affect the resilience and recovery potential of colonies [29, 32, 33]. Beekeepers often feed bees to increase overwintering survival (e.g.[37]) and several studies have shown that increases of flowers on farms, e.g. improved field margin management such as sowing of flower margins and strips also benefit other bee species [38] again highlighting the importance of improving forage availability for pollinators in agricultural landscapes [36].

The sensitivity to losses of in-hive bees' services is similar to the findings from Rumkee et al. [31] who also use BEEHAVE and find that overall the colony is more sensitive to loss of in-hive bees than foragers. The reason is that in-hive bees have received full energetic costs from the colony but not yet started to forage (and thereby returning some of the costs), so from an energetic point of view in-hive bees are the most expensive life stage to loose [31]. Here, in-hive bees were not lost, but as they could not do their job brood was lost and the energetic cost of raising brood rose substantially. There is some evidence that varroa mites make in-hive bees less active in brood care [39], and our Brood Care scenarios show how that could add to the damage varroa mites cause honeybee colonies.

In the sunflower scenarios, where exposure happened during late summer, the colony level impact coincided with the exposure period. However, in the oilseed rape scenarios, where exposure happened in spring, the colony level impacts increased after exposure had ceased and peaked approximately one month after exposure. We therefore recommend that in field studies colony assessments should continue at least one month after exposure finishes.

We here show that sublethal effects have the potential to have similar impact at the colony level as direct mortality. However, actual effects on individual bees will depend on the exposure concentrations they experience. So while many laboratory and semi-field studies indicate neonicotinoids have the potential to cause sublethal effects on individuals behaviour at high doses [21, 23], these studies were conducted with a contaminated sugar solution artificially fed to the bees. Another study shows that when bees are foraging naturally no impact on foraging activity or duration is observed [35]. There are different study types, which can be used to study sublethal effects on foragers in the field. RFID studies show whether number and duration of foraging flights are changed [23, 25, 26, 35]. However, RFID studies do not inform whether the foragers are active or just spending more time immobile, which would have lower energetic costs and therefore more likely less colony level impact. Harmonic radar studies will inform whether the bees are active and where they go in the landscape [21], but it is very labour intensive and only one bee can be studied at a time.

Nonetheless, as honeybees are social insects, effects on individual bees do not necessarily translate into effects on colonies as honeybees have many compensatory mechanisms (e.g. [27, 34]). For instance, Henry et al. detected effects on individuals, but this did not translate into colony level impacts because the colony could compensate [40]. Moreover, our simulations indicate that sublethal effects may have very different colony level impact depending on the type and timing of the effect.

Therefore, colony assessments are necessary to establish whether sublethal effects detected for individuals are ecologically relevant or not.

There are several field studies that have investigated the effect of neonicotinoid treated crops on naturally foraging bees. A field study with clothianidin treated canola (oil seed rape) shows no impact on bee mortality, worker longevity, brood development, colony weight change, honey yield or over-wintering success [41]. Another large field study with clothianidin treated oilseed rape seed [42] also finds no impact on honeybee colonies as did [43] in extensive field studies with thiamethoxam. Similarly, [35] find no link between neonicotinoid residues and colony performance and [44] using Hill's epidemiological criteria found no evidence that neonicotinoids are driving honeybee decline. So while this analysis using BEEHAVE indicated that sublethal effects may have the potential to create substantial impact on the colony, it does not appear from field studies so far conducted, that the exposure following the use of neonicotinoid seed treatments is sufficiently high to induce such effects under realistic conditions of use.

Our simulations indicated that if such sublethal effects were present at ecologically relevant levels, it would be detected in field studies if observation is continued for at least a month after exposure ceases. EFSA [45] recommends continuing observation for 2 brood cycles after exposure, which covers approximately 6 weeks after end of exposure. EPPO [46] suggests that assessments may be continued for longer intervals, e.g. to assess colony development over additional brood cycles if initial effects are seen. So study protocols and regulatory guidelines already exist, which recommend methods that would cover situations where colony level impacts occur after exposure finished.

We conclude that while sublethal effects on individual bees may be difficult to detect and quantify in field studies, ecologically relevant effects would be clearly visible in colony assessments provided observation continues for one month after end of exposure.

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

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Data Availability—In the supplementary material we provide extra figures, description of the changes to BEEHAVE and, if possible, the implementations of new versions of the model. To run the model versions provided would require download of the freeware Netlogo (http://ccl.northwestern.edu/netlogo/). Data, associated metadata, and calculation tools are available from the corresponding author (pernille.thorbek@syngenta.com).

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Figure 1. Colony level impact of simulated sublethal and lethal effects from exposure during oilseed rape bloom (Oilseed Rape Scenario workers in third year of exposure). The Y-axis show total number of workers, the X-axis day in the year and the dotted lines the exposure period, the horizontal panel shows the permanent distance between colony and forage patch. The line colours show the effect types, which in all cases were doubled relative to the control (for description see main text).

Figure 2. Effect on brood of simulated sublethal and lethal effects for colonies exposed during oilseed rape bloom (Oilseed rape scenario larvae in third year of exposure). The Y-axis show total number of larvae, the X-axis day in the year and the dotted lines the exposure period, the horizontal panel shows the permanent distance between colony and forage patch. The line colours show the effect types, which in all cases were doubled relative to the control (for description see main text).

Figure 3. Effect on colony weight of simulated sublethal and lethal effects during oilseed rape bloom (Oilseed Rape Scenario in third year of exposure). The Y-axis show colony weight (adults + brood+ pollen stores + honey stores), the X-axis day in the year and the dotted lines the exposure period, the horizontal panel shows the permanent distance between colony and forage patch. The line colours show the effect types, which in all cases were doubled relative to the control (for description see main text).

Figure 4. Effect of landscape mitigation on changes to colony dynamics caused by simulated sublethal and lethal effects for colonies exposed during oils seed rape bloom (Oilseed rape Landscape Mitigation scenarios for tripled Handling Time). The graph shows colony dynamics in third year of exposure. Mitigation forage patches were at different distances from the colony. Handling time was tripled and mitigation patches had 10% of the nectar and pollen in the main patch (which was placed 1000m from the colony); the mitigation patch was only present during exposure. The Y-axes show total number of workers, larvae, pollen stores and honey stores, the X-axis day in the year and the dotted lines the exposure period. The line colours show the mitigation level (for details see main text).

Figure 5. Effects on colony size of simulated sublethal and lethal effects for exposure during sunflower bloom (Sunflower Scenario workers third year of exposure). The Y-axis show total number of workers, the X-axis day in the year and the dotted lines the exposure period, the horizontal panel shows the permanent distance between colony and forage patch. The line colours show the effect types, which in all cases were doubled relative to the control (for description see main text).

Figure 6. Effect of landscape mitigation on changes to colony dynamics caused by simulated sublethal and lethal effects for colonies exposed during sunflower bloom (Sunflower Landscape Mitigation for Handling Time scenarios third year) with mitigation forage patches at different distances from the colony. Handling time was tripled and mitigation patches had 10% of the nectar and pollen in the main patch (which was placed 1000m from the colony); the mitigation patch was only present during exposure. The Y-axes show total number of workers, larvae, pollen stores and honey stores, the X-axis day in the year and the dotted lines the exposure period. The line colours show the mitigation level (for details see main text).

Figure 7. Mitigating effect of increasing levels of alternative unexposed forage (nectar and pollen) for exposure during sunflower bloom (Sunflower Landscape Mitigation scenarios for Handling Time third year of exposure). Handling time was tripled and distance was 1000m for main forage patch and 50m for mitigation patch, which was only present during exposure. The Y-axes show total number of workers and honey stores, the X-axis day in the year and the dotted lines the exposure period. The line colours show the mitigation level applied (for details see main text).

Table 1. Summary of effects on total number of workers (i.e. in-hive bees + foragers). Max difference: the maximum % difference between control and treatment any time during the 3 yr simulations. Difference 3rd yr: % difference between control and treatment at end of 3rd year. Time to recovery (TtR): number of days from exposure period ended to treatment groups had reached 99% of control for at least 3 consecutive days; NR: no recovery; 0: no effect at end of treatment period.

		Effect type			
Distance	Effect measure	Brood care	Disorientation	Handling time	Direct mortality
		Oilseed rape			
50 m	Max difference	-37%	-3%	-29%	-18%
	Difference 3rd yr	+1%	+1%	+1%	-1%
	TtR (d)	74	36	68	68
500 m	Max difference	-39%	-14%	-31%	-26%
	Difference 3rd yr	+3%	+5%	+2%	+0%
	TtR (d)	97	74	87	84
1000 m	Max difference	-55%	-13%	-21%	-26%
	Difference 3rd yr	-12%	+1%	-1%	0%
	TtR (d)	NR	101	106	226
		Sunflower			
50 m	Max difference	-2%	-2%	-2%	-12%
	Difference 3rd yr	0%	+1%	+2%	+0%
	TtR (d)	0	0	0	12
500 m	Max difference	-5%	-2%	-6%	-12%
	Difference 3rd yr	+2%	+3%	+2%	+2%
	TtR (d)	0	0	0	11
1000 m	Max difference	-9%	-5%	-10%	-19%
	Difference 3rd yr	+1%	-1%	-7%	-9%
	TtR (d)	13	0	NR	NR

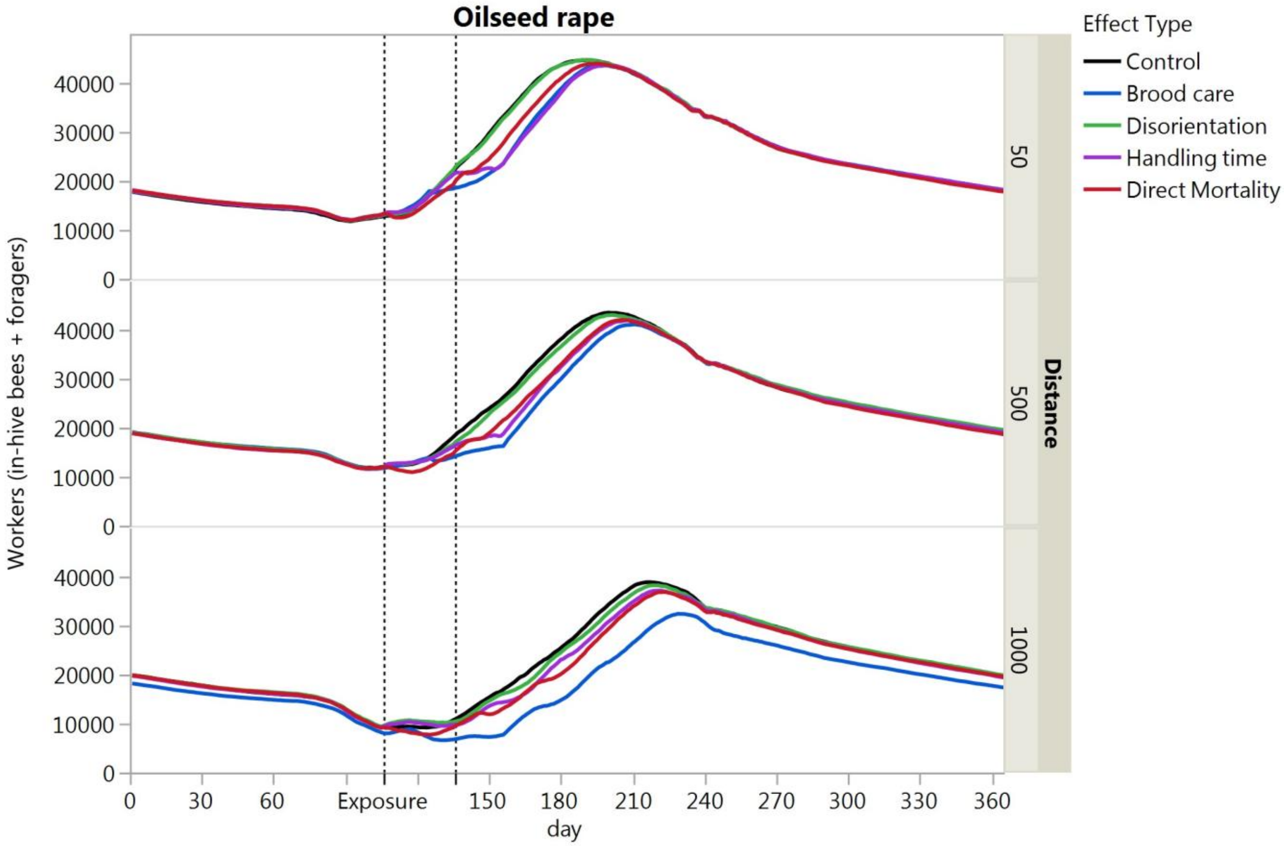
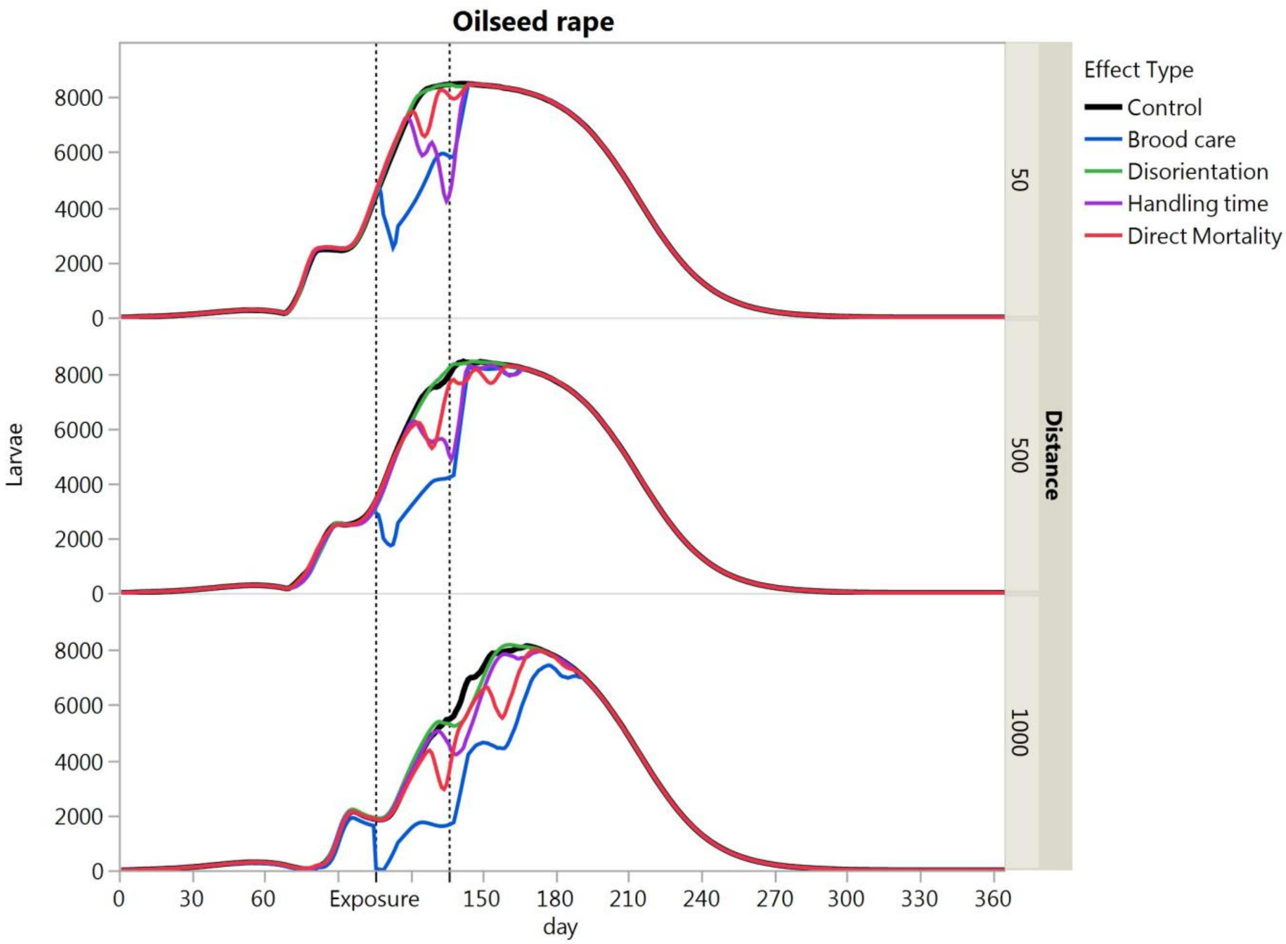
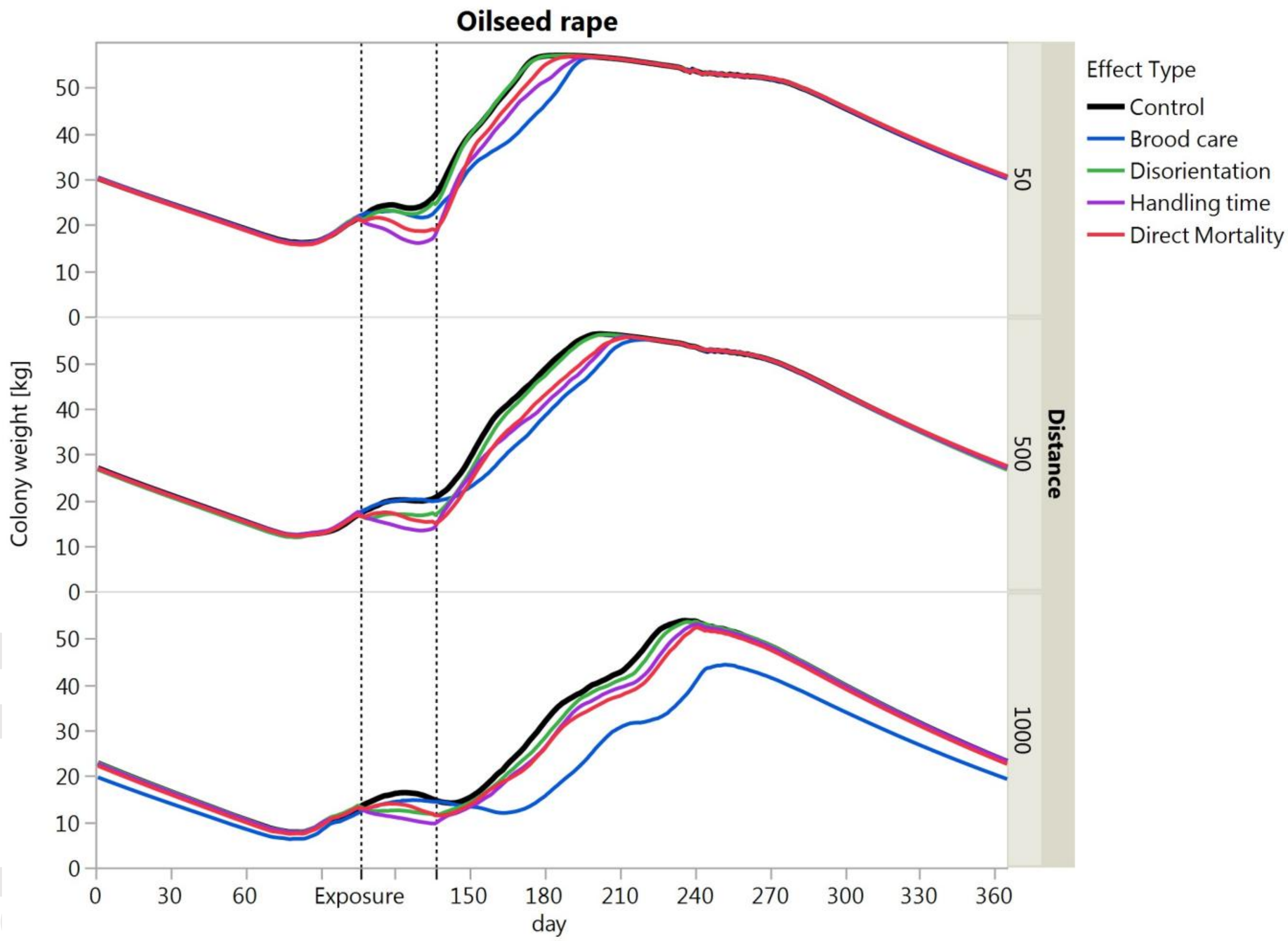

Oilseed rape
Effect Type
Control
Brood care
Disorientation
Handling time
Direct Mortality
Workers (in-hive bees + foragers)
40000
30000
20000
10000
0
50
500
1000
Distance
0
30
60
Exposure
150
180
210
240
270
300
330
360
day

Figure 1



Oilseed rape
Effect Type
Control
Brood care
Disorientation
Handling time
Direct Mortality
Larvae
day
Distance
50
500
1000
Exposure

Figure 2



Oilseed rape
Effect Type
Control
Brood care
Disorientation
Handling time
Direct Mortality
Colony weight [kg]
day
Exposure
Distance
50
500
1000

Figure 3

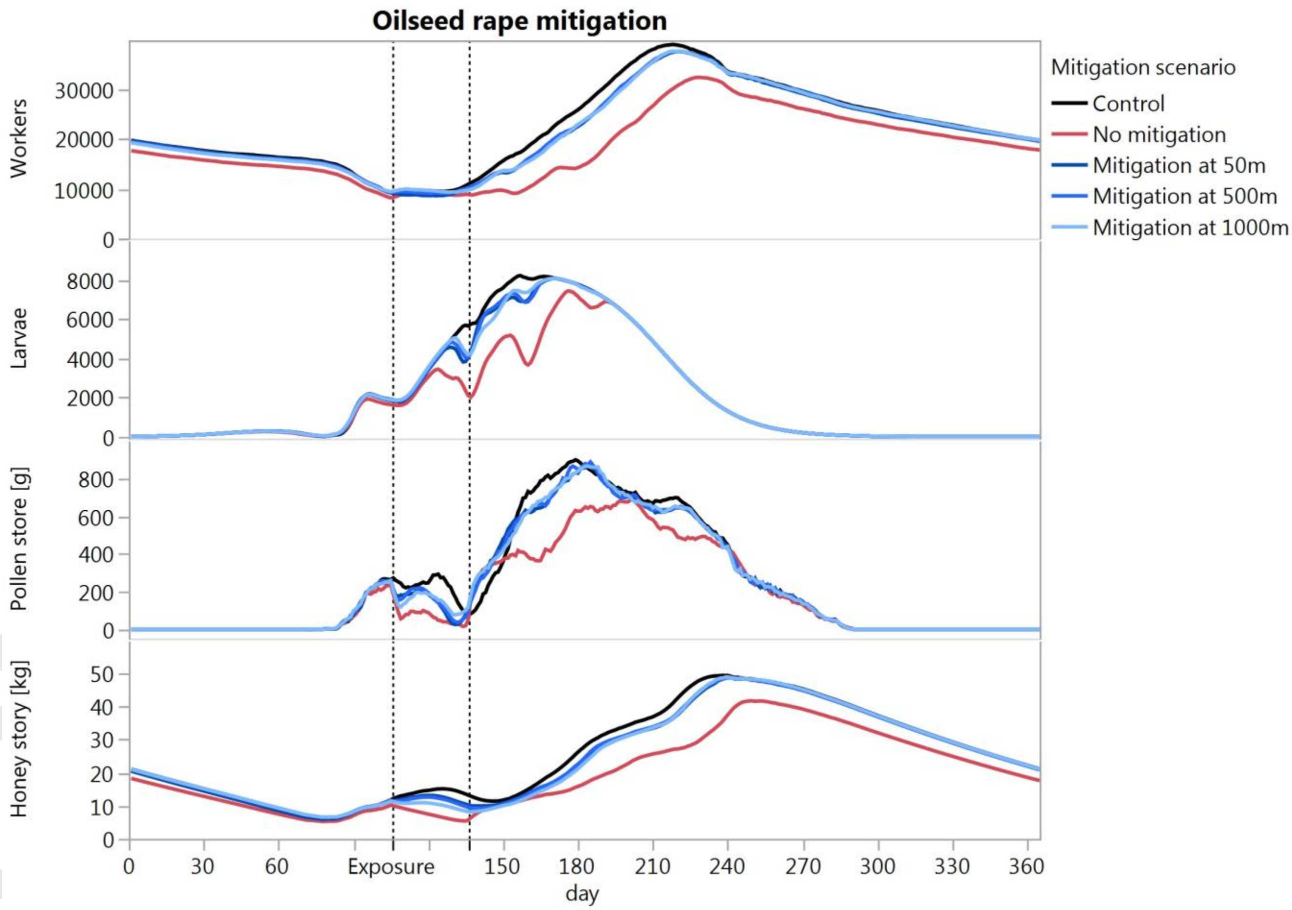

Oilseed rape mitigation
Mitigation scenario
Control
No mitigation
Mitigation at 50m
Mitigation at 500m
Mitigation at 1000m
Workers
Larvae
Pollen store [g]
Honey story [kg]
Exposure
day

Figure 4

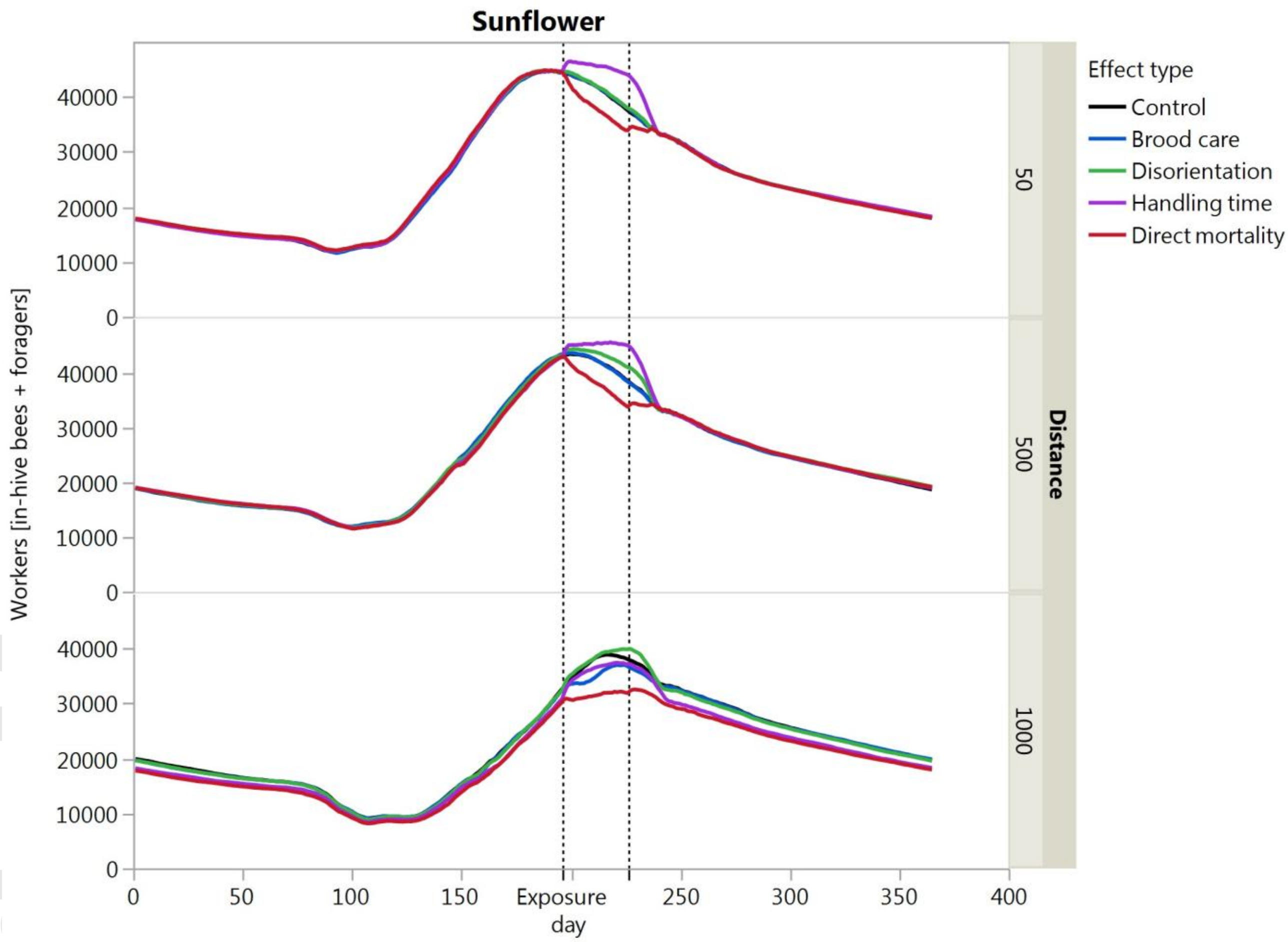

Sunflower
Effect type
Control
Brood care
Disorientation
Handling time
Direct mortality
Workers [in-hive bees + foragers]
Exposure day
Distance
50
500
1000
0
10000
20000
30000
40000
0
50
100
150
250
300
350
400

Figure 5

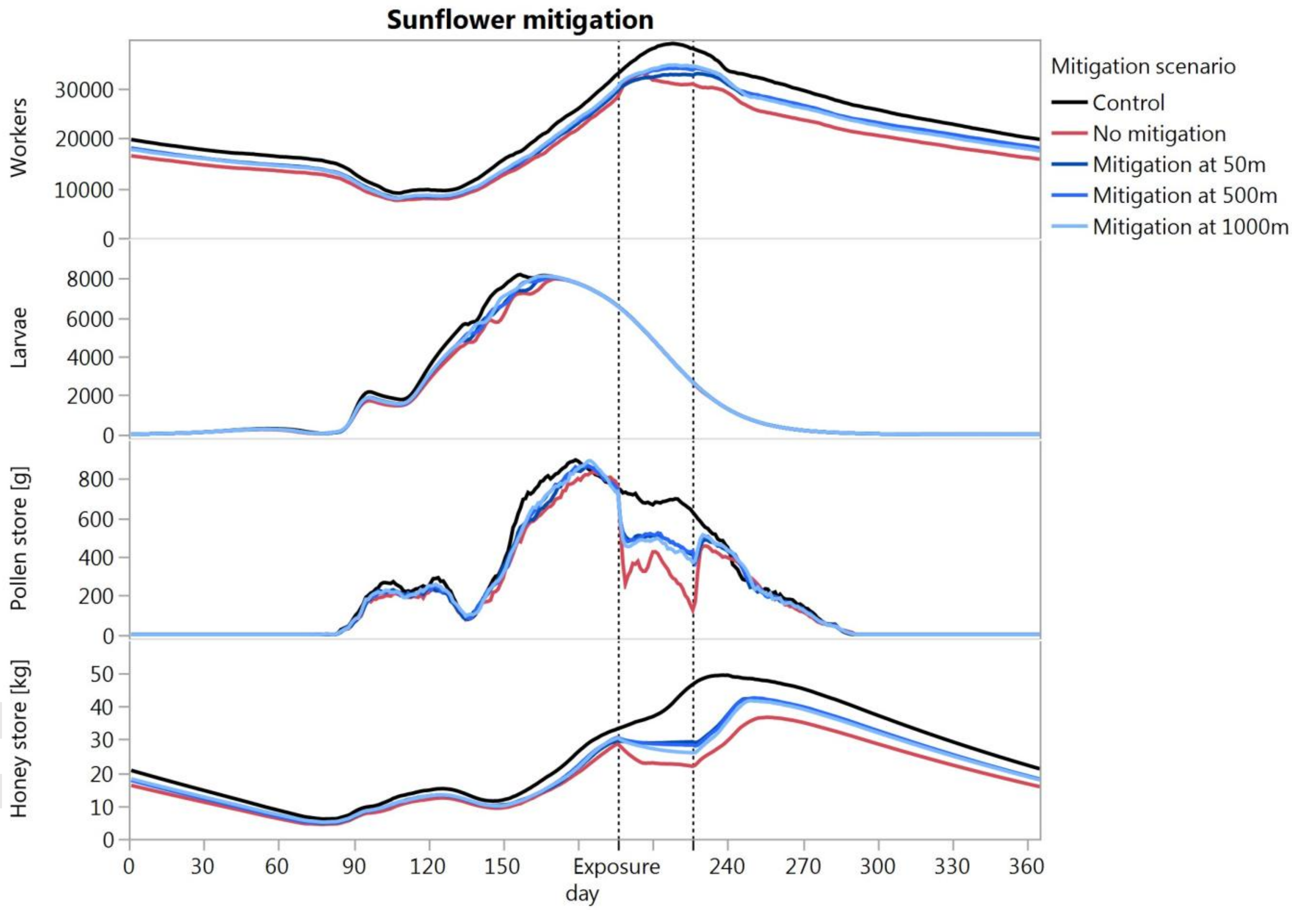
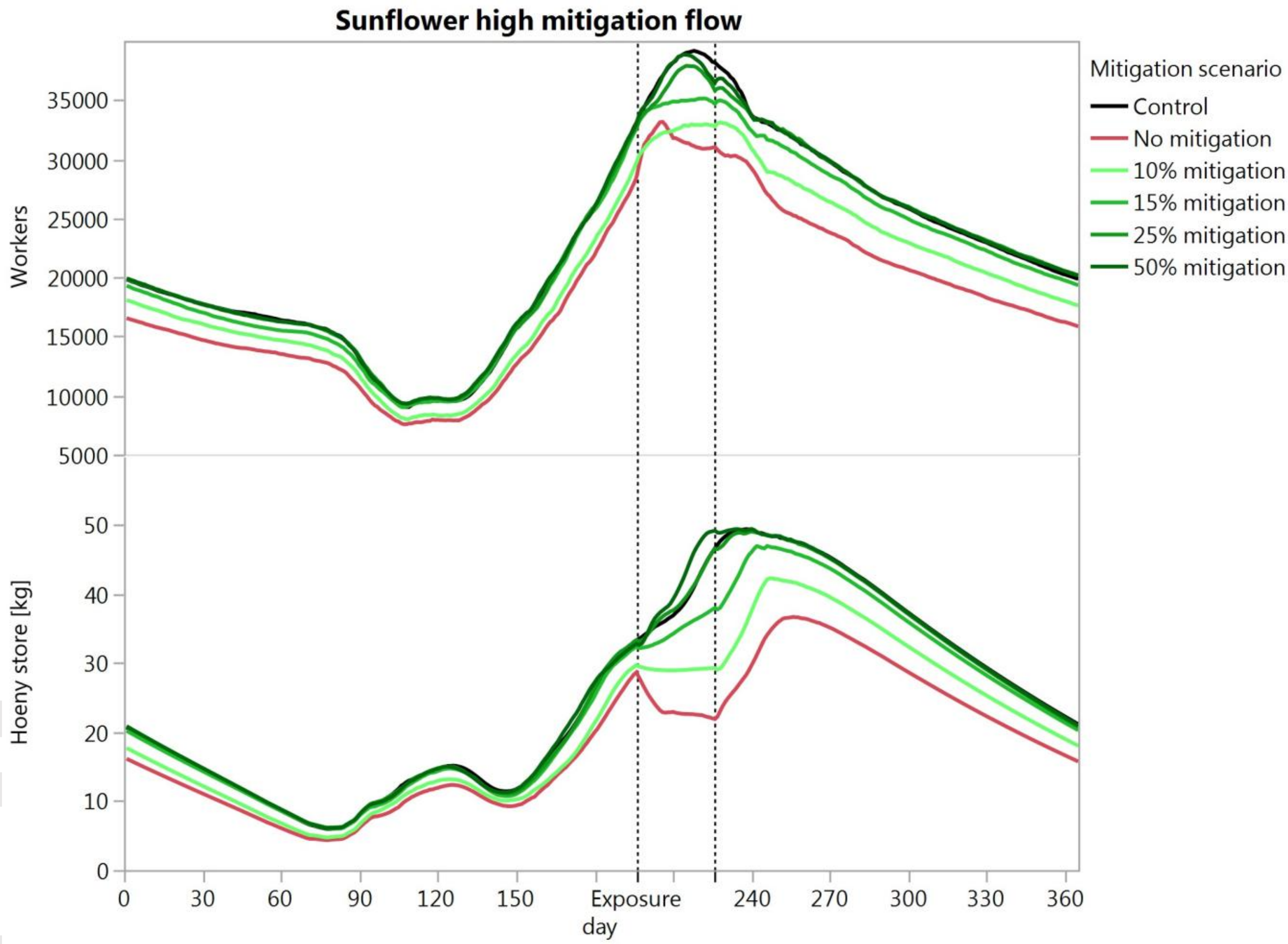

Sunflower mitigation
Workers
30000
20000
10000
0
Larvae
8000
6000
4000
2000
0
Pollen store [g]
800
600
400
200
0
Honey store [kg]
50
40
30
20
10
0
0
30
60
90
120
150
Exposure
240
270
300
330
360
day
Mitigation scenario
Control
No mitigation
Mitigation at 50m
Mitigation at 500m
Mitigation at 1000m

Figure 6



Sunflower high mitigation flow
Workers
Hoeny store [kg]
Mitigation scenario
Control
No mitigation
10% mitigation
15% mitigation
25% mitigation
50% mitigation
Exposure
day

Figure 7