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Effects of brood termination rate on colony viability – A BEEHAVE modelling study how timing, magnitude and duration of effects determine colony strength

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Abstract

The brood termination rate (BTR) investigated in higher-tier bee brood studies for plant protection product risk assessment is the determinant of honey bee (*Apis mellifera* L.) mortality during development from egg to adult. It influences colony strength, and in turn pollination services, hive products and colony viability. According to the EFSA Bee GD (2013), a honey bee colony is regarded viable, if at least 5000 worker bees are recorded prior to hibernation. We investigate how magnitude and duration of effects on the BTR affect the strength of honey bee colonies before overwintering and therefore viability. For this purpose, we modified and applied BEEHAVE, a computer model to simulate honey bee colony dynamics. Our modifications allowed for in silico representations of higher-tier bee brood studies under semi-field conditions with the option to follow bee colony dynamics until the end of the season. We have found that bee colonies are rather resilient to an increased BTR, such that under common experimental conditions, the number of brood cells as well as the colony size can recover over time. Yet, if BTR was above ≥ 70% (approximately the effect size caused by the reference item fenoxycarb) for a long period of 20 days or the brood study was started late in the season (1st August), recovery was slow. Nevertheless, only if modelled experiments were started late in the season (1st August), there was a risk of colony sizes below 5000 worker bees before winter (31st October). This risk was found for treatments and control due to the seasonally reduced egg laying rate of the queen. Compared to the control the risk was only relevantly enhanced, if BTR was ≥ 70% for the entire brood cycle.

Keywords: Honey bees, BEEHAVE, model simulation, brood termination rate, colony strength

Introduction

The brood termination rate (BTR) investigated in higher-tier bee brood studies (such as the feeding studies according to Oomen *et al.* 1992 or Lückmann & Schmitzer 2019 as well as semi-field studies according to OECD GD 75 2007) for plant protection product risk assessment (RA) is the determinant of honey bee (*Apis mellifera* L.) mortality during development from egg to adult. It influences colony strength, and in turn pollination services, hive products and colony viability. According to the EFSA Bee GD (2013) a honey bee colony is regarded as viable and strong enough for successful overwintering and subsequent development to a vital colony in the following year, if at least 5000 worker bees are recorded prior to hibernation. We investigate how magnitude and duration of effects on the BTR affect the strength of honey bee colonies before overwintering and therefore viability.

Material and methods

The honey bee model BEEHAVE (Becher *et al*. 2014) simulates the colony dynamics in a bee hive in relation to the resource availability in the landscape. We adjusted the model (version 2016) to

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explicitly analyse the impact of BTRs at different magnitudes, at different starting times in the season and for different durations on the amount of bee brood shortly after the start of brood termination and on the colony strength after two brood cycles as well as shortly before overwintering (Table 1). In this context, we aimed at keeping the modelling study qualitatively comparable to typical field or semi-field toxicity test scenarios.

Table 1 *Full factorial design of parameter variation for BEEHAVE simulations.*

Parameter	Values	Rational
BTR [%]	Control	Default values from BEEHAVE
	0	No BTR (even less than control)
	20	Average BTR of the control in field experiments*
	30	Average BTR of the control in tunnel experiments*
	50	-
	70	Average BTR of positive reference in tunnel experiments**
	100	BTR removing entire brood
Starting time of BTR modification [day of year]	1st June	Typical start date of tunnel experiments
	1st July	Typical start date of tunnel experiments
	1st August	Late start date of tunnel experiments
Duration of modified BTR [d]	10	Covering egg and larval feeding stage
	20	Covering almost one full brood cycle; duration of effects caused by the insect growth regulator fenoxycarb **
Time of measurement [days after application]	5	Covering development of egg and young larvae
	20	Covering almost one brood cycle
	44	Covering two brood cycles
	31st October	Before hibernation

* see Lückmann & Tänzler (2020); ** see Lückmann et al. (2023)

We accounted for test scenarios in terms of firstly setting a fixed day in the season, when the test started. On this day, we adjusted the size of the colony to approximately 6000 bees, which is the minimum colony strength according to OECD GD 75 (2007). Simulation models as well as natural systems respond to abrupt artificial changes, such as the reduction of colony sizes. To ensure natural colony composition and to minimize disturbance of the colony dynamics, we applied a stepwise approach. Firstly, we started BEEHAVE with slightly modified standard settings (without *Varroa* infestation or bee keeping activities such as honey harvesting, colony merging, bee feeding – see Table 2). The modifications helped to isolate our study target, the effect of BTR, from other complex processes and their interactions. Secondly, we calculated the reduction factor *rf* as the ratio of 6000 bees and the simulated total hive size at BTR start. Thirdly, the number of in-hive individuals at each age stage was multiplied with *rf*, which proportionally reduced the number of larvae, pupae, nurse bees and drones. To adjust the number of forager squadrons, a proportion of *rf* squadrons was randomly selected. Also honey and pollen stores were adapted by multiplication with factor *rf (*see also Preuss *et al.* 2022*)*. The procedure resulted in hives of approximately 6000 worker bees. In order to account for variability in beehive dynamics, we repeated the procedure eight times. Each replicate provides slightly different initial conditions for the BTR simulation experiments. Practically the replicates can be considered as different test hives in an experiment.

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Table 2 *Changes to BEEHAVE default settings*

Parameter	BTR analysis	Default settings
allowreinfestation	FALSE	TRUE
dronebroodremoval	FALSE	TRUE
efficiencyphoretic	0	0.05
honeyharvesting	FALSE	TRUE
mergeweakcolonies	FALSE	TRUE
n_initial_mites_healthy	0	10
n_initial_mites_infected	0	10
rand_seed	0	1
stopdead	TRUE	FALSE
swarming	No swarming	Swarming (parental colony)
treatmentday	0	180
treatmentduration	0	20
x_days	151	161

BTR was modelled as a daily egg mortality at the day of egg-laying. This approach ignores that in reality bee brood might be terminated at any point during the twentyone-day brood cycle, and a later termination is connected to a higher loss of nursing investment. However, in the context of plant protection product application, the assumption was deemed appropriate, because compounds predominately affect the uncapped young larvae (see Lückmann & Schmitzer 2019). We considered effects of lasting increased BTR by applying the egg mortality for several days. This is a conservative assumption in the context of plant protection product applications, because usually effect strength declines over time.

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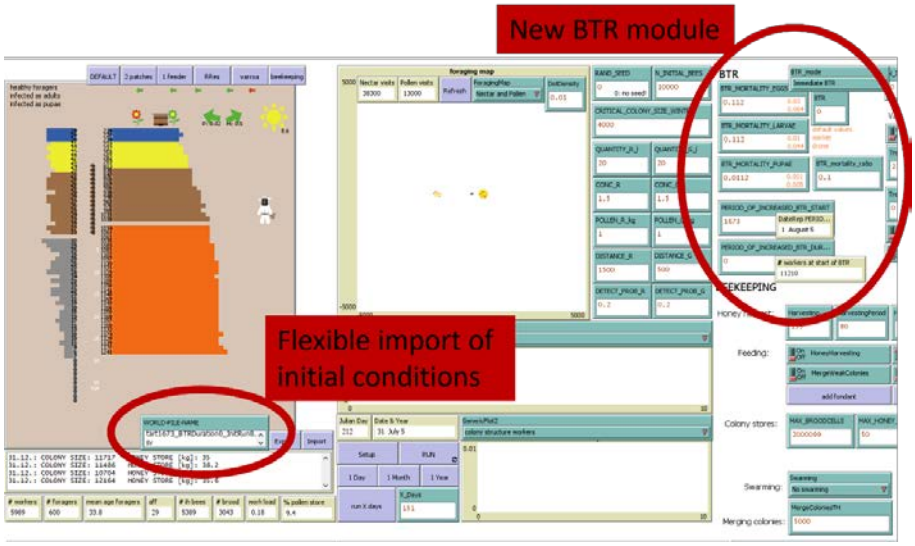

Figure 1 *Screenshot of the modified BEEHAVE software. Red circles indicate the new brood termination rate module and the possibility to flexibly import pre-defined initial conditions via NetLogo world files.*

The simulation experiments were conducted in Netlogo (Wilensky 1999), the programming platform, in which the BEEHAVE software is implemented. Netlogo provides the tool 'Behaviour space', which supports parameter sweeping, *i.e.*, the program automatically varies specified parameters. We varied the start and duration of a BTR period as well as the BTR strength in a full-factorial design. The tested parameter values are described in Table 1. In the model simulations, we accounted for natural variability in two ways: (1) We used the eight replicates of starting conditions for the simulation experiments. (2) We repeated the experiment for each parameter set 10 times, to account for the variability during and after the experiment. This resulted in 8 × 10 = 80 replicates per parameter set.

As the 'Behaviour space' cannot directly accommodate for the variation of initial conditions, we inserted the varied parameters in the so-called NetLogo world, using statistical software R (R Core Team 2022). Each of the modified worlds was then imported by 'Behaviour space' and automatically processed.

For the analysis, we monitored the simulated amount of bee brood shortly after the start of the impact *(i.e.*, after 5 and 20 days) and the colony strength after two brood cycles (*i.e.*, 44 days) as well as shortly before overwintering (*i.e.*, at the end of October). Finally, we estimated the proportion of colonies with a colony size lower than 5000 workers at the end of October. The proportion was calculated as the number of replicates with colony size below 5000 on 31st of October divided by the 80 replicates.

Results

Impact on the brood

Simulated BTR reduced the number of brood cells (including open and capped cells) in a colony in an effect size staggered way (Figure 2). With higher BTR, the brood cell reduction was stronger.

Five days after the start of the BTR manipulation period (Figure 2, left panel), the absolute reduction of the median number of brood cells started from different levels, amounted approximately 4000 cells (difference between minimum and maximum number) and was indedependent from the season, when the increased BTR was simulated (upper to lower rows).

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At day 20 (Figure 2, right panel), the number of brood cells was still decreased with increasing BTR. Yet, seasonality and duration of BTR increase became more influential. With the shorter BTR increase period of 10 days (Figure 2, left figure column) and low to intermediate BTR strength (*i.e.*, 20 to 50%), the number of brood cells was similar to control (except at a study start on 1st August), which reflects that the colony fully recovered the brood losses within the ten days after the end of the brood reduction period. The potential for recovery was slightly higher when BTR increased earlier in the season compared to an increase starting at the beginning of August.

For the longer BTR period (20 days – Figure 2, right figure columns), the reduction of the number of brood cells was stronger when brood was counted after 20 compared to an evaluation after 5 days. Thus, brood reduction accumulated.

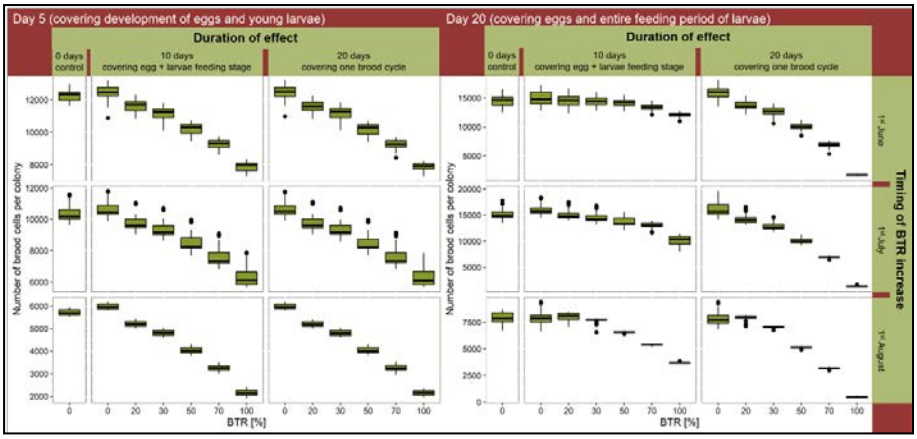

Figure 2 *Sensitivity of the number of brood cells to BTR counted at day 5 (left pannel) and at day 20 (right panel) of the onset of changed BTR. Figure columns show BTR increase periods of 10 days (left column) and 20 days (right column). Figure rows indicate the start day of the BTR increase period (upper row 1st June, middle row 1st July, bottom row 1st August). Boxes show median (central line) and span the 50% quantiles, whiskers roughly indicate the the 95% confidence interval and dots the extremes. Note that the number of brood cells is slightly higher with a BTR of 0% than in control, which reflects that natural brood mortality was ignored in BTR manipulations compared to control.*

Impact on the colony strength

BTR increase could affect colony size over intermediate time periods and to a minor exent until the end of season (Figure 3).

At day 44, *i.e.*, two brood cycles after the start of BTR increase (Figure 3, left panel) the number of worker bees was considerably lower than in control, if for a period of 20 days BTR was intermediate or high, independently when the increase of BTR started. A shorter period of 10 days of BTR increase reduced the number of worker bees only, if BTR increased intermediately or highly late in the season (1st August). Instead, almost no effects were found for a 10-day increased BTR starting at 1st June or 1st July, which means that colonies recovered from even severe impacts within two brood cycles (1.5 months).

Consequently, hardly any effects from periods of increased BTR up to 70% on colony size were found by the end of the season (1st October). Only from BTRs of 100% over 10 days slight differences occurred and from high BTRs of ≥ 70% over 20 days the colonies did not recover.

If studies started 1st June or 1st July, all colonies displayed a strength ≥ 5000 workes bees, independent the magnitude and duration of increased BTRs. But colonies remained particularly small

in the control and the treatment if studies started 1st of August, due to the seasonally reduced egg laying rate of the queen.

There was a risk that colony sizes dropped below 5000 worker bees (dotted line in Figure 3), which is an assumed realistic threshold for viable hibernation (see EFSA 2013). For studies which started 1st of June or July no colony displayed a strength below the value, irrespectively the size and duration of the BTR. For studies which started 1st of August, the proportion of colonies below this strength was low (*i.e.*, < 10%) for the control, if the BTR increase lasted for only 10 days (irrespective its magnitude) or if BTR increase lasted for 20 days but its magnitude was equal or below 30% (Table 3). Lower proportions at higher BTRs in these cases were due to to chance. However, the proportion of colonies with less than 5000 worker bees increased strongly up to 80% if the magnitude of the BTR was 50% or above for 20 days.

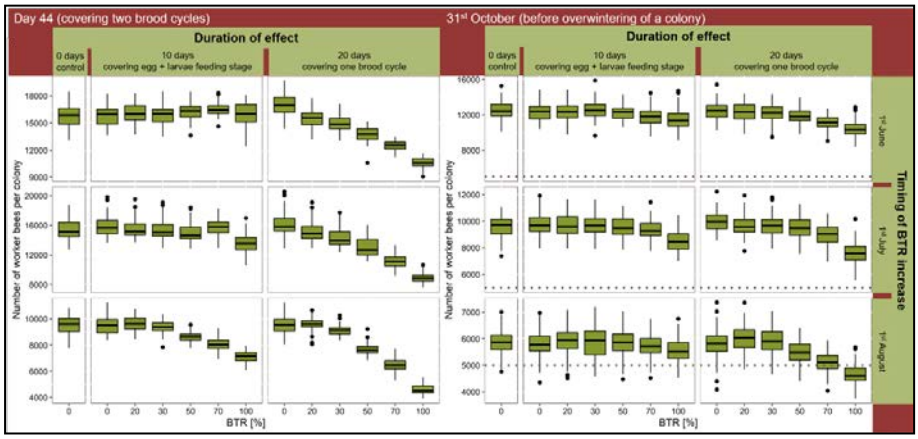

Figure 3 *Sensitivity of the number of worker bees to a BTR modification at day 44 of the onset of changed BTR (left panel) and on 31st October (right panel). Figure columns show BTR increase periods of 10 days (left column) and 20 days (right column). Figure rows indicate the start day of the BTR increase period (upper row 1st June, middle row 1st July, bottom row 1st August). Boxes show median (central line) and span the 50% quantiles, whiskers roughly indicate the 95% confidence interval and dots the extremes. The horizontal dotted line marks the threshold of 5000 worker bees for a viable overwintering colony size.*

Table 2 *Risk that colony size had dropped below 5000 worker bees before hibernation on October 31st.*

Starting date	BTR	Proportion of replicates with less than 5000 worker bees [%]	
		10 d BTR duration	20 d BTR duration
1st June	**all**	0.0	
1st July	**all**	0.0	
1st August	**CONTROL**	3.8	
	0	7.5	3.8
	20	7.5	2.5
	30	5.0	1.3
	50	8.8	13.8
	70	8.8	36.3
	100	8.8	80.0

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Discussion and Conclusion

The BTR is currently one of the most important endpoints in higher tier (semi-) field studies for assessing plant protection product risk to honey bee brood according to Oomen et al. (1992), Lückmann & Schmitzer (2019) and OECD GD 75 (2007). However, the duration of these tests is restricted to roughly 1 month and does not cover an entire summer season. Thus, the meaning of measured BTRs in terms of colony dynamics and viability for the whole season is not well understood.

With the aim to understand the impact of different BTR magnitudes and durations on hibernation viability of bees, we in silico mimicked an OECD GD 75 (2007) test but continued simulations until onset of the hibernation period on 31st of October. We simulated the impact of an increase in BTR on the size of honey bee colonies across time scales with a customized version of the well established hive simulator BEEHAVE (Becher *et al.* 2014). In a full-factorial sensitivity analysis, we considered aspects of BTR strength and timing, as typical determinants of brood manipulation experiments.

During a period of increased BTR, we found quick exertion of effects, such that honey bee brood had strongly decreased after 5 or 20 days. Nevertheless, when the period of increased BTR ended, the impact on the brood ceased, and recovery of the hive started immediately. For example, 10 days after a 10-day period of weakly to intermediately increased BTR (*i.e.*, 20 to 50%), the number of brood cells had recovered to the control level.

However, if BTR was strong, even after the shorter 10-day period, the number of brood cells had not fully recovered. Particularly, later in the year, recovery seemed slower, probably due to the seasonally reduced egg laying rate of the queen. The seasonal pattern in the number of brood cells is also reflected in the number of worker bees one and a half months after the onset of BTR increase (*i.e.*, after two completed brood cycles).

Yet, if the intermediate or strong BTR duration already spanned over one brood cycle, the remaining brood cycle was insufficient to exert recovery at the level of worker bees. This can be understood in the extreme case of BTR = 100%, where brood of one complete cycle was terminated prematurely. Starting with the following cycle, brood cells were quickly filled, and this new brood developed normally. By the end of the second cycle, only the oldest of that new generation just matured and were counted as workers. Others were still in development. Therefore, compensation of adult mortality that occurred during the cycle, when no brood survived, can only be observed later (not shown here).

Hives usually recovered until the end of season to similar size as controls. Only at high BTR of ≥ 70%, rates known for highly toxic reference test compounds, minor effects still persisted.

Our results indicate that the timing of experiments is the most critical factor. Particularly, if the experiment was started late in the season (here 1st August), the colonies were small before hibernation. These small hive sizes were a result of the reduction of the colonies to 6000 worker bees at the beginning of the experiment late in the season, when egg laying of the queen has already been seasonally reduced. This combination of an initially small colony with low growth kept colonies considerably smaller than when experiments were started earlier in the season. That is why the preparation of new nucleus colonies normally takes place between April and beginning of July, which provides them enough time to develop to sufficiently large colonies before overwintering. Addtionally, bee keeping practice has demonstrated that summer brood interruption for subsequent

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Varroa treatment is a well know tool without impacting the colony strength before overwintering to a critical level (Büchler *et al.* 2020).

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