# BEEHAVE – Analysing the Significance of Increased Brood Termination Rate on the Colony Strength in Honey Bees

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SETAC EUROPE 33RD ANNUAL MEETING
30 APRIL - 4 MAY 2023 | DUBLIN, IRELAND + ONLINE

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The brood termination rate (BTR) investigated in higher-tier studies according to OECD GD 75 [1] for pesticide risk assessment is the determinant of honey bee (*Apis mellifera*) mortality during pre-imaginal development and thus influences colony strength. While the current EFSA Bee GD [2] indicates risk to honey bee colonies, if overwintering colony size in late autumn drops below 5000 bees, the revised draft EFSA Bee GD [3] defines that a reduction in colony size of up to 10% at all time after pesticide application compared to control hives is acceptable. By using the honey bee colony model BEEHAVE [4], we simulated colony dynamics following increasing BTRs to present the consequences of the new risk definition of > 10% colony size reduction.

## Material & Methods

The honey bee model BEEHAVE simulates colony dynamics in a bee hive in relation to resource availability in the landscape. The model was adapted to explicitly model the link between BTR at different timings, magnitudes and durations versus the colony strength in weekly intervals during two consecutive brood cycles [5 also for more details].

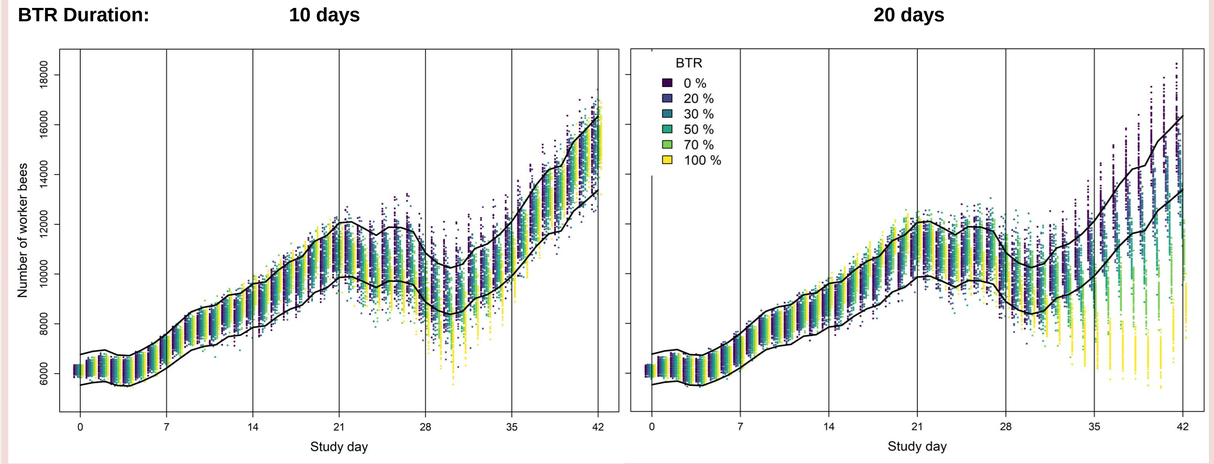
Duration of modified BTR levels: 10 and 20 days

Starting time of BTR modification: 1st June, 1st July, 1st August

BTR effect sizes: 0%, 20% = approx. control level in field studies [6], 30% = approx. control level in semi-field studies [6], 50%, 70% = approx. level for brood affected by the reference item fenoxycarb in semi-field studies [6] & [7], 100%.

Settings of the model:
Colony size at start of BTR increase: ca. 6000 bees (lowermost colony strength according to [1]); age class composition of workers and drones as well as food stores were adapted accordingly to minimize the disturbance of the colony. Replicates: 8 initial colonies, replicated for 10 times; n = 80.

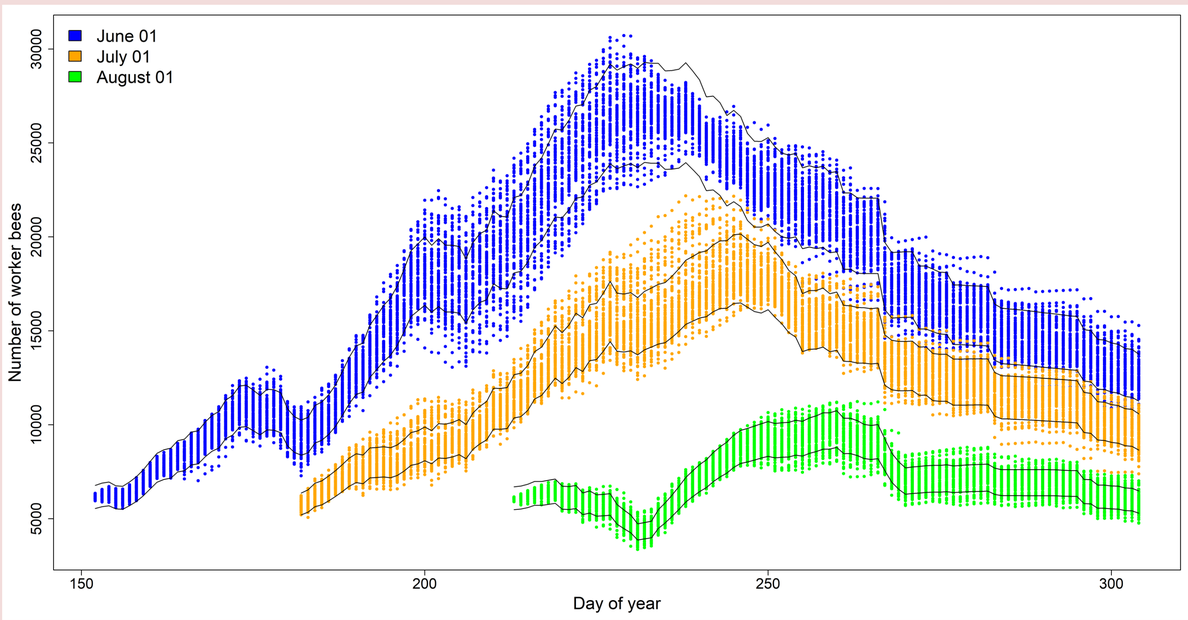
## Results: Colony strength




**Effects of different BTR levels (colours) and durations (left: 10 days, right: 20 days) during two brood cycles (42 days) for studies starting 1st of June**
Dots indicate simulation replicates. Upper and lower black lines mark the range of ± 10% around the daily mean control colony strength.

- Reduction in colony strength larger than 10% is likely for BTR ≥ 50%.
- Change of colony strength depending on time of bee counting.

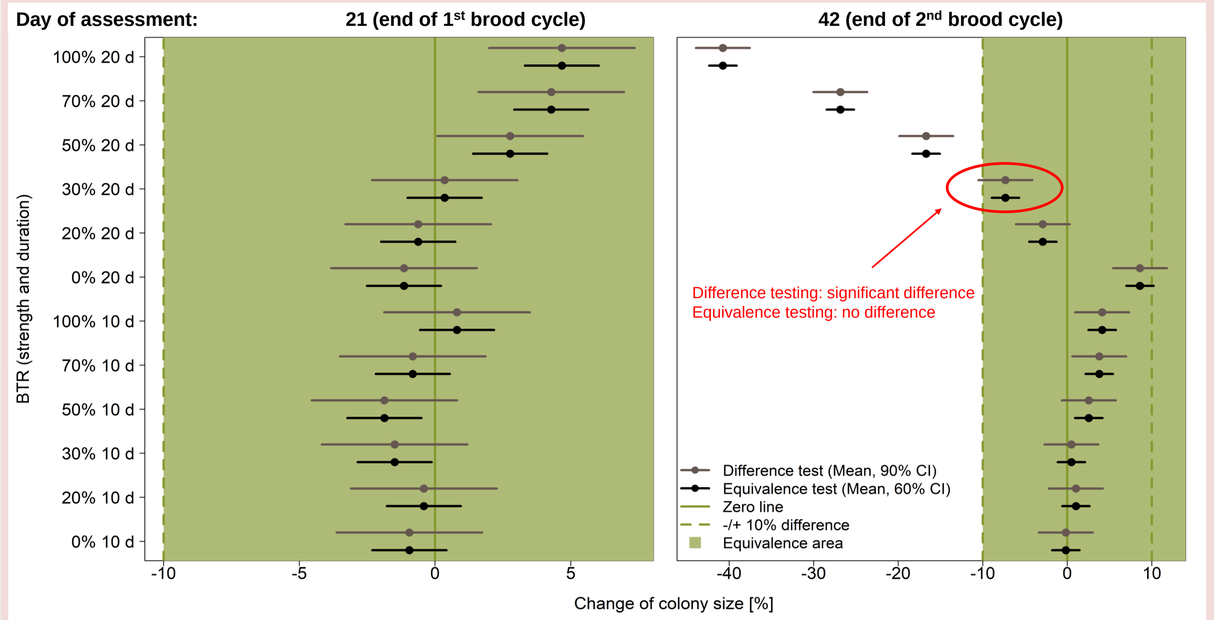
## Results: Control variability




**Variability of colony strength in control colonies**
Studies start beginning of June, July or August. Dots indicate simulation replicates. Upper and lower black lines mark the range of ± 10% around the daily mean colony strength. Time span: 1st June to 31st October.

- Colony strength varies over time due to seasonality. Colonies, starting earlier in the year reach higher maximal and overwintering sizes.
- Variability of ± 10% covers most of the simulated naturally occurring variability until end of October.

## Results: Difference vs equivalence testing




**Difference and equivalence testing of BTR effects on colony strength for studies starting 1st of June**
Reduced colony strength is significant according to [3], estimated by mixed linear modelling with variation among hives as random effect:
***Difference testing*** 90% confidence intervals (CI grey) include 0 (solid vertical line).
***Equivalence testing*** 60% CI (black) overlap the 10% size reduction (dashed -10% line).

- Significant negative effects found for BTR ≥ 50% at duration of 20 days.
- Difference and equivalence testing usually coincide in effect detection. In few cases the difference test seems more conservative.

## Conclusion & Perspectives

- Simulated natural variability of colony strength in control colonies ranges around ± 10%.
- Considering this variability as random effect in a linear mixed model isolates the effect size.
- Effects of BTR on colony strength can be positive or negative. At colony level (worker bees), BTR effects (acting on the brood) are delayed.
  Positive effects might result from reduced foraging and therefore lower mortality. Brood deprived colonies have reduced demands on pollen and nectar, and therefore lower foraging needs. Negative effects occur delayed, when reduced brood cohorts mature and cause a lack of workers.
- The newly suggested equivalence test performs in general similarly to standard statistical tests (difference testing).
- For the simulation analysis, equivalence testing was less conservative compared to the standard test in borderline cases.

**References:** **[1]** OECD (2007) GD on the honey bee (*Apis mellifera* L.) brood test under semi-field conditions **[2]** EFSA (2013) GD on the risk assessment of plant production products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 11. **[3]** EFSA (2022) Revised GD on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus spp.* and solitary bees). Draft update for public consultation. **[4]** Becher M, Grimm V, Thorbek P, Horn J, Kennedy P J & Osborne J L (2014) BEEHAVE: a systems model of honeybee colony dynamics and foraging to explore multifactorial causes of colony failure. J App. Ecol 51: 470-482. **[5]** Singer A, Lückmann J, Becher M, Jakoby O, Metz M (submitted): BEEHAVE and brood termination rate – A modelling study how timing, magnitude and duration of effects determine colony strength Julius-Kühn-Archiv. **[6]** Lückmann J & Tänzler V (2020) Honeybee brood testing under semi-field and field conditions according to Oomen and OECD GD 75: is there a difference of the brood termination rate? Julius-Kühn-Archiv 465: 91-95. **[7]** Lückmann J, Bluhm W, Kimmel S, Steeger T & Wilkins S (submitted): Brood termination rate in honey bees in two consecutive brood cycles, Julius-Kühn-Archiv.

Parts of this work were conducted within the VIBEE project (vibee-project.net) and financed by: