

Improved test design for the evaluation of semi-field studies with bumblebees (*Bombus terrestris*)

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INTRODUCTION

With the publication of the EFSA revised bee guidance document on the risk assessment of plant protection products announced for May 2023, standardised higher tier studies for bumblebees will be requested. In the first draft of this guidance (EFSA 2022), the endpoints colony growth (weight increase of colonies) and reproductive success (formation of new queens) at the end of colony development are required as endpoints for a risk assessment.

Between 2015 and 2019, the ICPPR Non-Apis Working Group developed a bumblebee semi-field study design and tested it in an inter-laboratory trial (Klein et al 2022). The final results showed that the test methods and endpoints cannot be adopted from established semi-field tests for honey bees. In particular, colony evaluation and the production of new queens caused difficulties.

Therefore, tier3 solutions started working with IPM Impact to develop a test design under GLP that meets the requirements of the revised bee guidance and monitors additional parameters of colony development. Minimally invasive assessment methods and new research and development (R&D) hives were used that do not interfere with the natural development of the colonies and the reproductive success.

BACKGROUND and DEVELOPMENT GOALS

Test issues of the Bumblebee ring-tests 2015 - 2019

- For the colony assessments, the colonies (and if necessary, the canopy covering the brood nest) were opened to count the number of worker females as well as brood cells. This influenced the development of the colonies to varying degrees. It led to a strongly divergent development or, in many cases, to the premature death of the colonies or no forming of new queens.

Goal → To find reliable assessment parameter that avoid opening hives and/or modifying hives for easier monitoring

- The variability of the delivered colonies led to a high variability in colony development, which made the final analysis of most developmental endpoints and of the reproductive success difficult.

Goal → To guarantee homogeneous bumblebee colonies in age structure and with same parentage.

- The production of young queens in the controls could not be reliably achieved at many test sites. The selection of the most homogeneous (in terms of size, structure and genetics) starter colonies and provision of good feeding sources during the whole field-phase (exposure and post-exposure site) were identified as main factors (Klein et al. 2022).

Goal → To guarantee reproductive success in control colonies by using “better” initial colonies.

R&D colonies and hives

Starter colonies:

- 1 mother queen (sister queens)
- Prepared brood nest
- 20 callows (young worker of the same age)

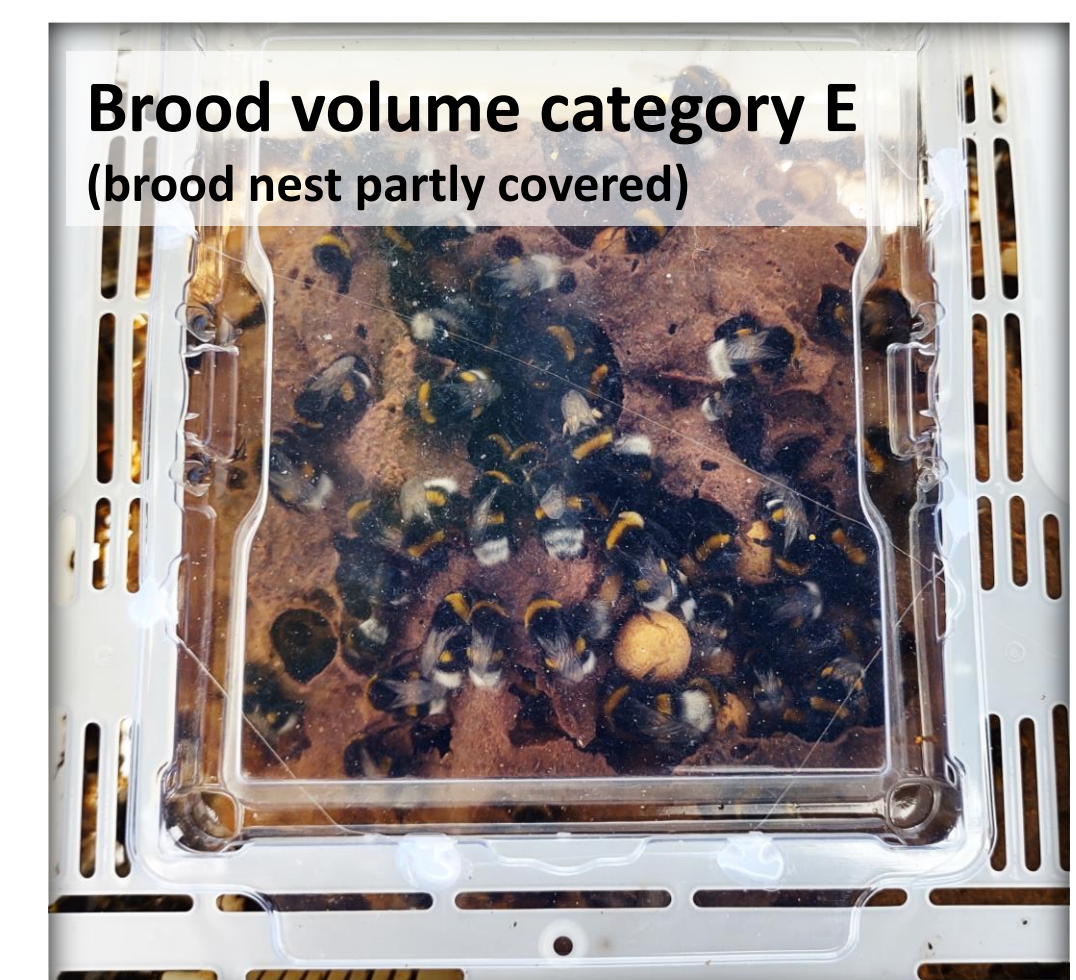
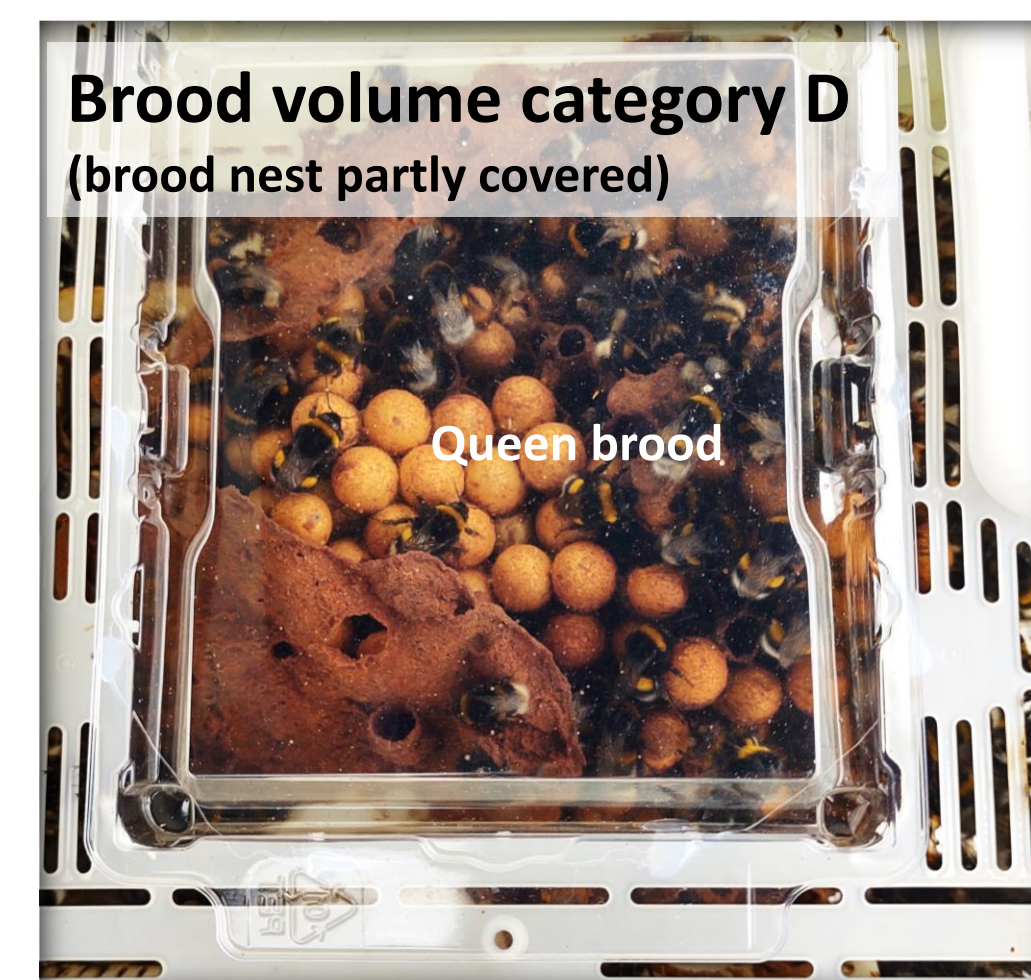
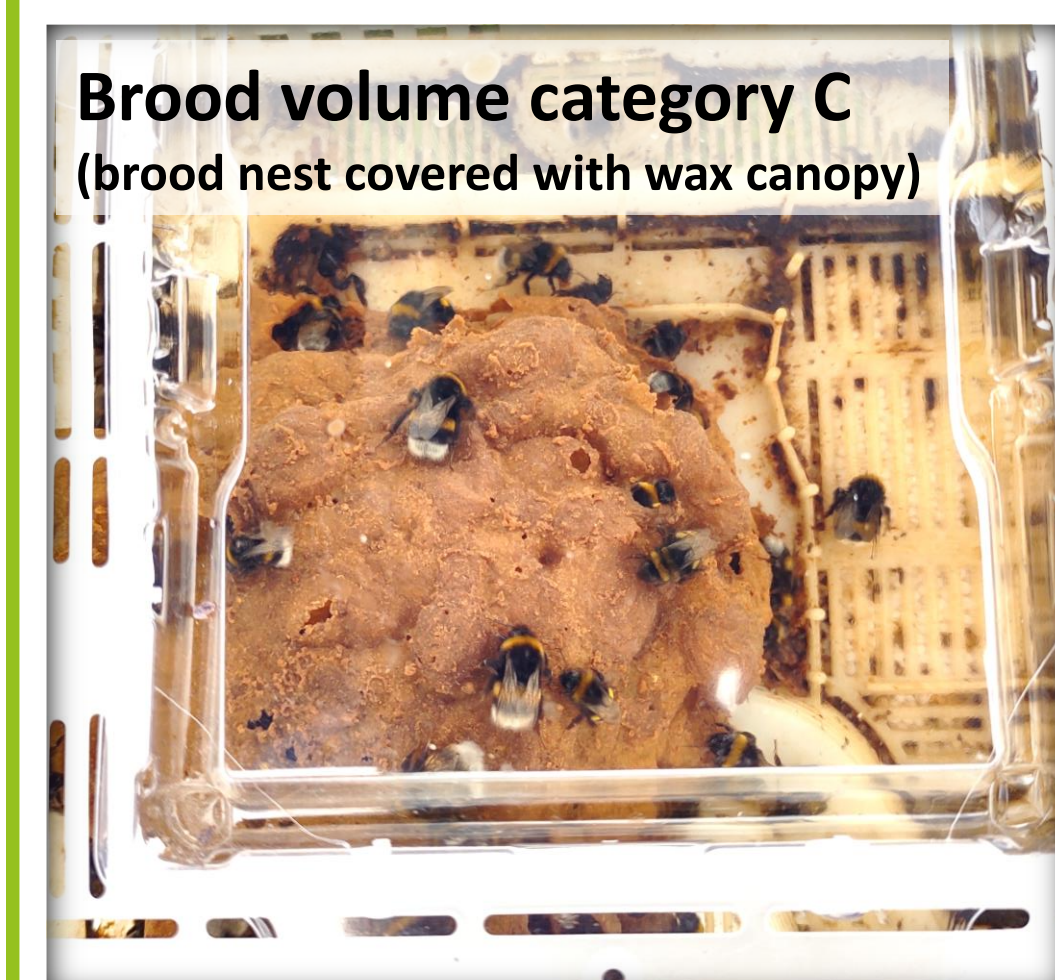
Bumblebee hive:

- Transparent window
- Modified nest area
- Queen logger at entrance

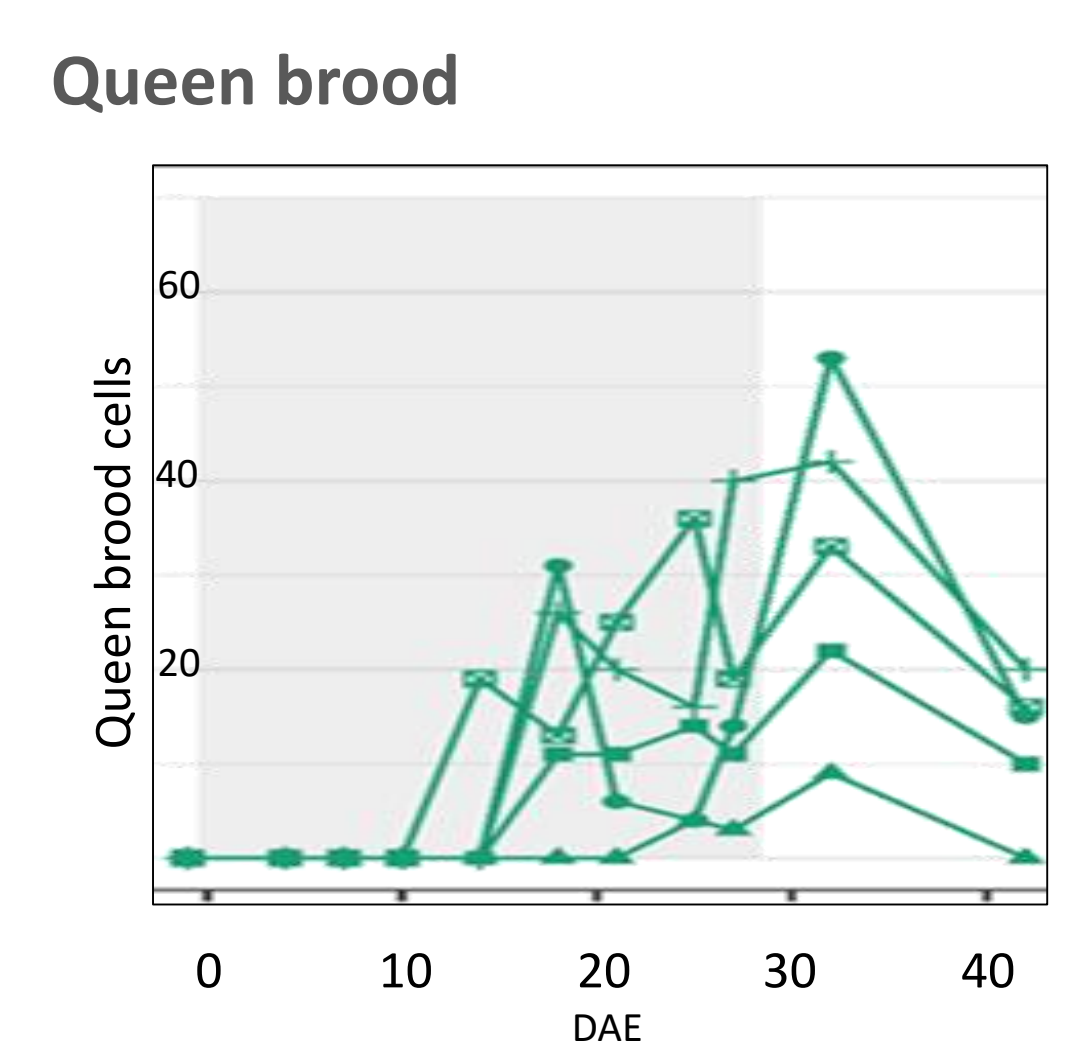
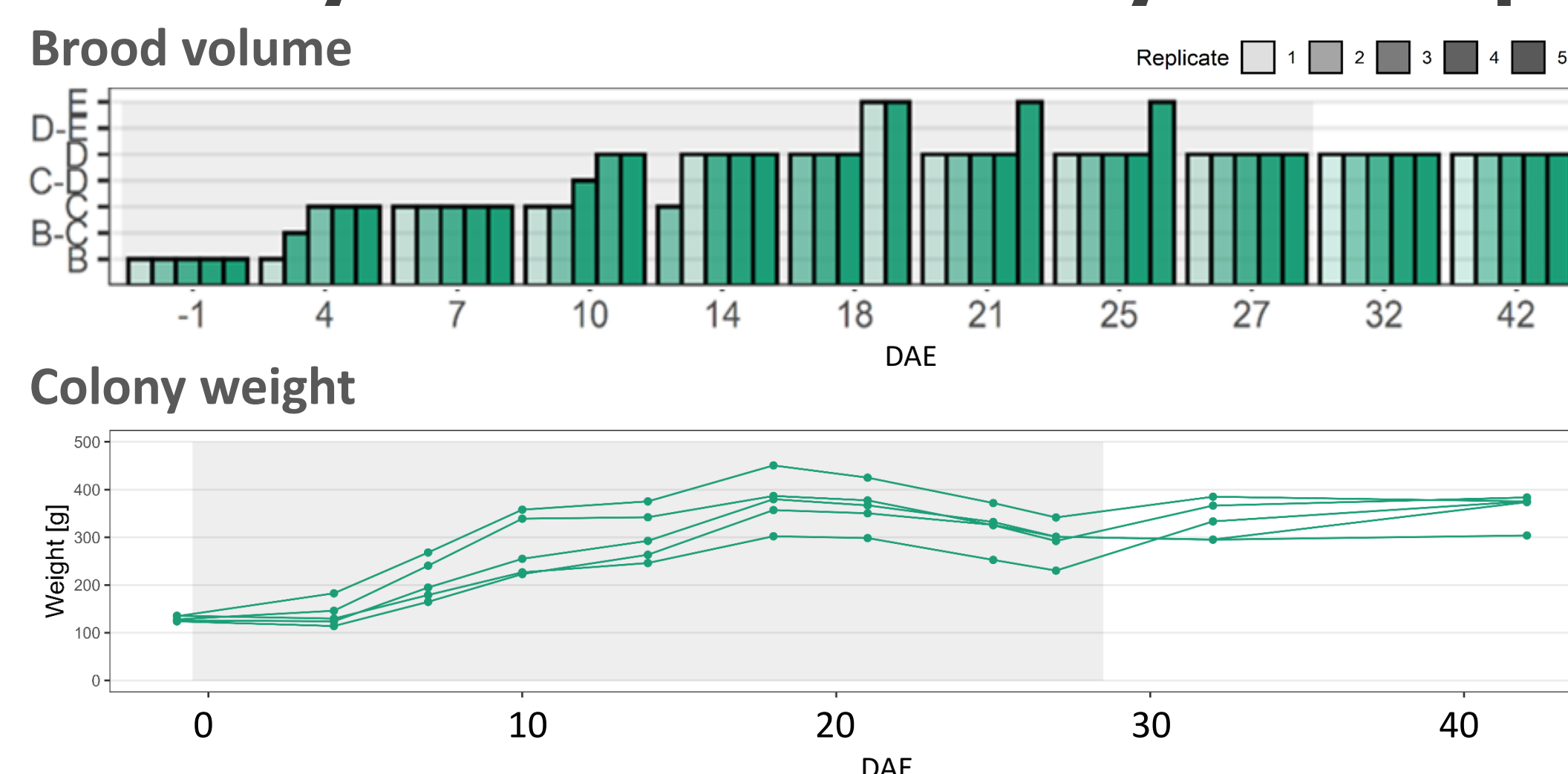
Brood volume category B



Additional assessment parameter: Brood volume



Result: Synchronised colony development



CONCLUSIONS

- With R&D colonies, the variability in the colony development could be significantly reduced and new queens hatched in all control colonies towards the end of the trial.
- The modified hives significantly facilitated colony assessment without compromising the development of the colonies.
- The endpoints for the risk assessment of bumblebees (EFSA 2022, Cabrera et al. 2016), reproductive success and the colony development could be determined with high certainty with our testing design.

