



# Long-term exposure in semi-field studies of the Large Earth bumblebee (*Bombus terrestris*)

Markus Persigehl<sup>1</sup>, Guido Sterk<sup>2</sup>, Florian Ballhaus<sup>1</sup>, Mareike Beinert<sup>1</sup>, Britta Kullmann<sup>1</sup>

<sup>1</sup> tier3 solutions GmbH, Kolberger Str. 61-63, 51381 Leverkusen, Germany

<sup>2</sup> IPM Impact, Gierkensstraat 21, 3511 Hasselt, Belgium

## INTRODUCTION

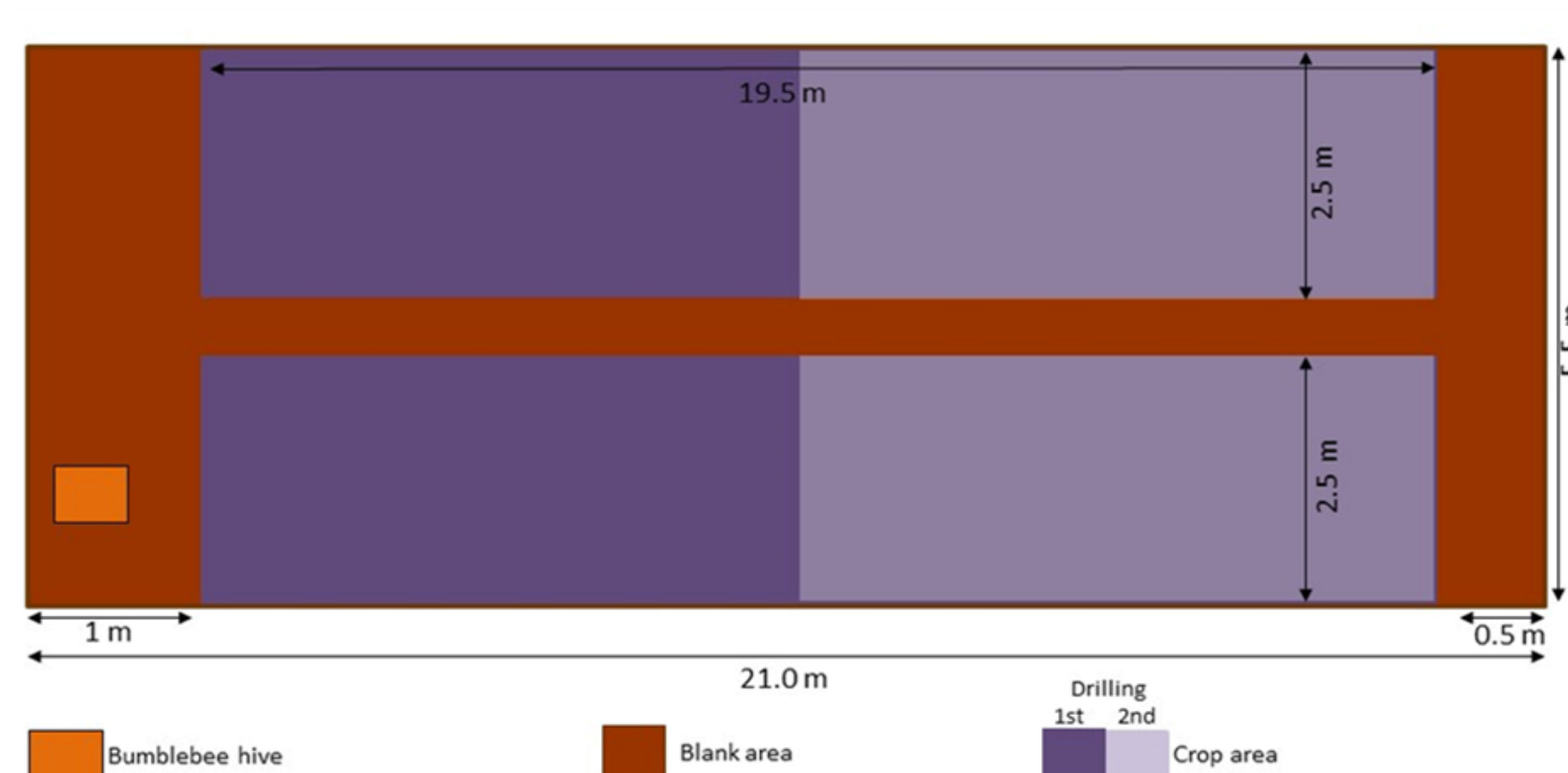
Biological plant protection products (biopesticides), such as fungi, bacteria or nematodes, are playing an increasingly important role in integrated pest management. Biopesticides, like chemical plant protection products (PPP), must be authorised according to (EC) 1107/2009 in Europe. But most test systems developed for chemical PPPs do not work for biopesticides, because their mode of action is usually quite different (Borges et al. 2023). For a reliable risk assessment, tests must therefore always be adapted to the respective biology of the test item and its mode of action. In the example presented, an exposure period of seven days, as for chemical PPPs in semi-field effect studies, is not considered sufficient for the risk assessment of an insectivorous entomopathogenic fungus, as the exposure of pollinators, such as bumblebees, increases over time due to the growth of the fungus in the crop. The experimental design presented was developed in consultation with regulatory authorities.

## STUDY GOALS

- Enable controlled long-term exposure (4 weeks) of bumblebee colonies to BioControls
- Monitoring of colony development and reproductive parameter (offspring queen reproduction)

## Method 2: Tunnel set-up

- 5 tunnels (= replicates) for control and test item + 2 tunnels toxic (dimethoate) reference



- Long flowering through controlled irrigation and successive flowering strips.

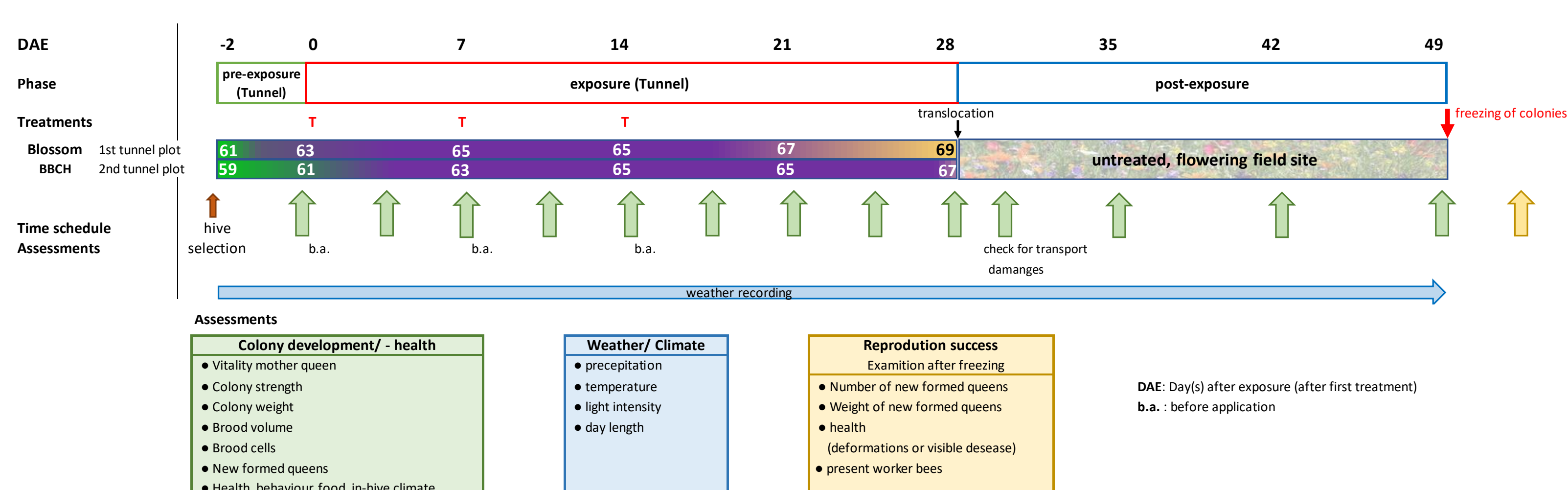
## Method 3: R&D hives

- Maximum uniformity of initial colonies: 1 mother queen + 10-25 callows
- New developed hives to reduce the impact of the biological assessments



Transparent panorama-window facilitates monitoring

## Method 1: Time schedule



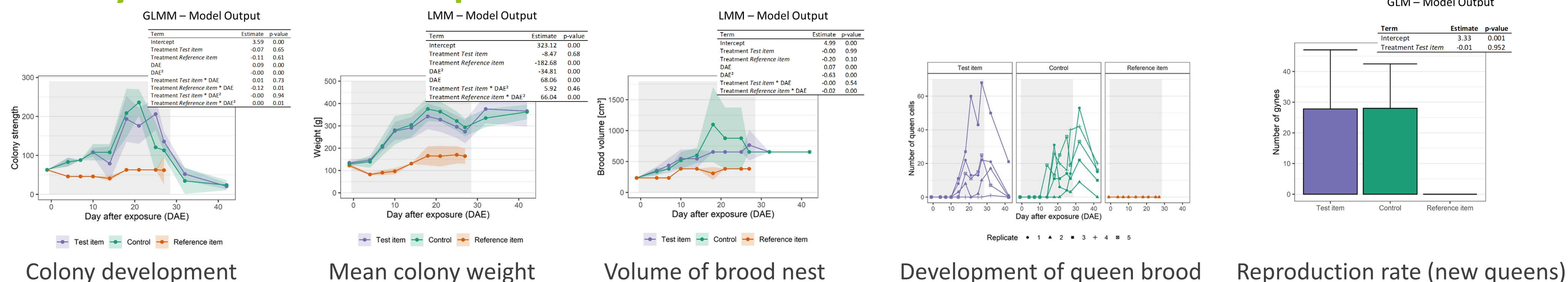
## Method 4: Assessment parameters (endpoints)

- Presence/vitality of mother queen
- Colony strength
- Colony weight
- Brood volume
- Number of queen-brood cells
- Number of new formed Queens (gynes)

➡ **Minimum invasive assessment methods**, facilitated by R&D hives, should allow monitoring without unnecessarily disturbing the colonies, which minimises premature colony loss and ensures a high likelihood of reproduction of new queens.

- Further recordings: Colony health, abnormal behaviour, food stores, in-hive climate, weather conditions (non-GLP)
- Generalized linear mixed models used to evaluate the colony development and reproductive power between treatment and control bumblebee colonies

## Data analysis of relevant endpoints



## RESULTS & CONCLUSIONS

- Bumblebees were exposed to the test design for four weeks under controlled semi-field conditions without limiting colony development.
- The test design and the R&D hives ensured maximum synchronized development of all bumblebee colonies and the formation of young queens in 100 % of the control colonies.
- The recorded data were reliably analysed by GLMMs.

