III. Test methodology - laboratory, semi field, field, etc.

Assessment of brood development and index calculations

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Abstract

According to European requirements, regulatory testing and risk assessment on honeybee brood is required in cases where colonies are exposed to treatments that could induce forager bees to bring back residues to the hive in nectar or pollen. The impact of chemicals to honeybee brood can be assessed both in laboratory and field conditions.

The existing brood testing methods are available only for laboratory conditions (Aupinel and al., 2007) and for semi-field conditions (OECD n°75, 2007). However, the impact on honeybee brood is easily been assessed in a field test, combining recommendations of EPPO n°170 or French CEB n°230 for the field part and the OECD 75 for the brood assessment part.

Currently brood tests under laboratory conditions are managed according to the OECD document n°75 'Guidance document on the honey bee (*Apis mellifera* L.) brood test under semi-field conditions'. According to this guideline, data on the brood development and growth stages in at least 100 marked cells over 22 days are collected.

In the evaluation the test results allow calculations of indices:

- the brood termination rate (failure of the brood development),
- the brood index (measure of the larval development)
- the compensation index (indicator for recovery).

However results from field conditions and from semi-field conditions may differ because of the colony behavior or because of external conditions such as climate and enclosure under tunnels. Although all results are valid, in some cases the control and the reference item provide unexpected results. Such data with mortality in the control are usual in beekeeping practice but not suitable for calculation to the indices. This occurs mainly under semi-field conditions. Hopefully the OECD document n° 75 indicates "*Specific statistical analysis… are still under development*". It is so reasonable to provide information for deciding how to use the results.

The eggs laid by the queen develop into larvae, then pupae and then honeybee, emerging in a precise time sequence. Individuals deviating too much from the normal time sequence are disqualified. For evaluating the different brood stages of single marked cells, the recorded growth stages are recalculated into values from 0 to 5.

At least 100 cells containing an egg are selected on a dedicated area on a first observation timing (the Brood Fixing Day = BFD)

If a cell does not contain the expected brood stage during the period from 5 days to 16 days after the Bood Fixing Day (BFD+5 to BFD+16), the cell has to be counted as 0 at the assessment day and also on the following days. Most of the time, when the brood development is abnormal this justification will eliminate the cell from the further calculation. However, in a limited number of situations, it is possible that the brood development is quite normal although the expected stage is not attained. Beekeepers observations confirm that honeybees may deviate from the theoretical time sequence. In such a case index calculations are not adapted to the colony development.

Adapted formulas allow considering this normal brood development when conditions require. In this way raw data (brood development) need to be transformed to adapted data (complementary data = to be used for the index calculation).

For instance an *Excel* formula takes into account the early stage of capped cells:

- By considering the initial conditions of eggs then larvae into cells (IF/OR in the software) and the expected development (AND/OR in the software) with the realized development in each individual cell (Brood evolution data /Complementary data).
- In order to increase the data significance we think it is reasonable to use adapted formulas from a bee keeper point of view. Brood tests in-field or semi-field cannot easily be replicated; therefore we find it necessary to increase the significance of collected data in single studies.

Keywords: brood, OECD guidance document

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