2.4 Reference data project 2014 – 2015 for the assessment of control data

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Introduction

For the assessment of side effects of plant protection products on honeybees (*Apis mellifera* L.) in the official risk assessment procedure experimental data will always be evaluated in relation to the untreated control. In 2014 and 2015 control data were assessed for different investigations. These data should help to better interpret experimental data. Various approaches were run to assess the natural removal rate of honeybee colonies: the brood development was observed by photo assessments and laboratory larval tests (OECD GD 239) were run.

Material, Methods and Results

Photo assessments for brood development observation

In May and July/August 2014 (ascending and descending colony development) cells containing eggs were fixed on brood combs and were daily photographed until hatch. One observation series was started with eggs of defined age (caged queen) another series was started with eggs of undefined age.

The emergence rate was uniform at 92.7% (n = 410 eggs with defined age, n = 700 eggs with undefined age) in May. But the removal rate was significantly higher in July/August and showed a higher variability. 87.6% of the eggs with defined age (n = 428) and 34.9% of the eggs with undefined age (n = 651) did not develop successfully until hatch.

By the daily photo assessments for the observation of the development of eggs with undefined age it could be shown that the highest increase of mortality occurred during open breeding stages (26.6% until day 5 of the observations). It was concluded that this is the most sensitive phase during the natural breeding within the colony.

In order to minimize negative influences by daily photo assessments 250 eggs were marked in 6 colonies each in July and August 2015 and were photographed only on defined days (brood fixing day (BFD) 0, +5, +10, +16, +22 [1]) for the observation of the development until hatch. The removal rate was between 2.0% and 10.0% (median 4.8%) in July and between 3.2% and 28.4% (median 12.8%) in August.

Natural emergence rate (2014 daily photographed and 2015 on BFDs)

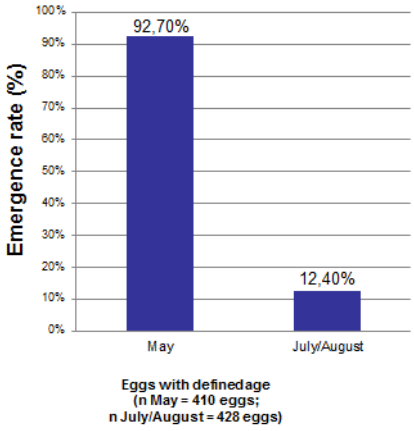


Fig. 1 Queen caged for 24 h

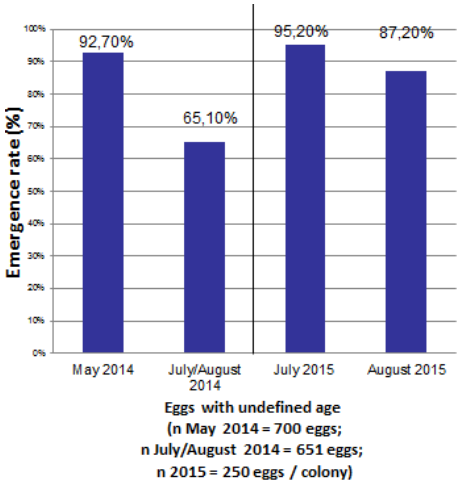


Fig. 2 Brood combs with eggs with defined age

Brood development (photographed every 24h ± 15 min, queen caged for 24 h)

Only by looking on the photos it was not possible to determine the age of the eggs. But the duration of every single brood stages could be evaluated by analyzing the photos: egg phase: 3 days, larval phase 5 ± 1 days, pupal phase: 12 ± 1 days, emergence on BFD 20 ± 1.

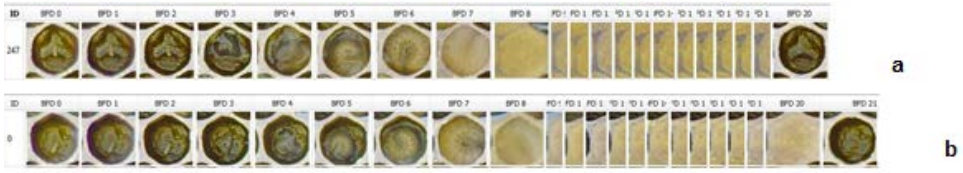
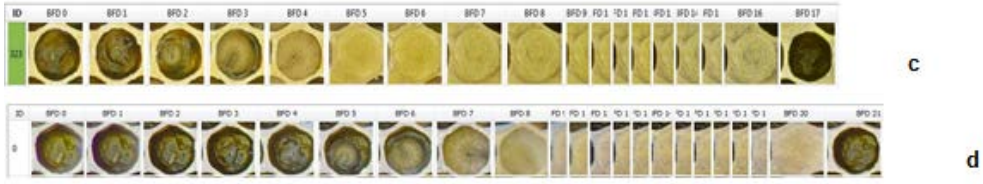


Abb. 3 a - d: Photo assessments for the observation of the brood development



On BFD 5 ± 1 young to old larvae were expected [1]. But the assessments showed captured cells. On BFD 17 (= BFD 16 + 1) these cells were already empty (eggs with undefined age). Until BFD 21 every observed brood cell was empty (n = 2189 eggs, eggs with defined and undefined age). Captured cell which were found on BFD 22 ± 1 possibly resulted from refilled cells.

Laboratory larval test – variability of control mortality and factors which influence the mortality

The natural variability of the emergence rate in the artificial rearing of bee-brood was investigated in the laboratory larval test [2]. In 14 trials with 10 plates à 48 larvae each 5616 larvae were reared in total from April to July 2014. From grafting on day 1 until day 6 the larvae were fed with a special diet and were observed until emergence on day 22.

The average emergence rate on day 22 was 70% (min. 14%, max. 98%). But only 53% of the plates with 48 larvae achieved an emergence rate of > 70 %. There was observed a sudden mortality increase during the pupation phase (day 8 until day 15). On this data base the pupation phase was

identified as the most critical phase of the test method. The colony effect (larval origin), the seasonal effect (test start during the season) and different ages of test individuals at the time of the grafting (young L1-larvae, older L1-larvae [3]) were investigated as potential negative factors on the success of the test method. The results shown in figure 4 indicate that larvae of different origins vary with regard to their tolerance to develop successfully in an artificial rearing system. A pre-test should be carried out prior to the actual laboratory testing for the selection of suitable "larvae colonies".

In the trials in June 2014 poorer results for the successful emergence were found than in the other test periods (figure 5). For this reason a seasonal effect could not be neglected.

In tests which were started with older L1-larvae as test individuals more reliable emergence rates were found (figure 6).

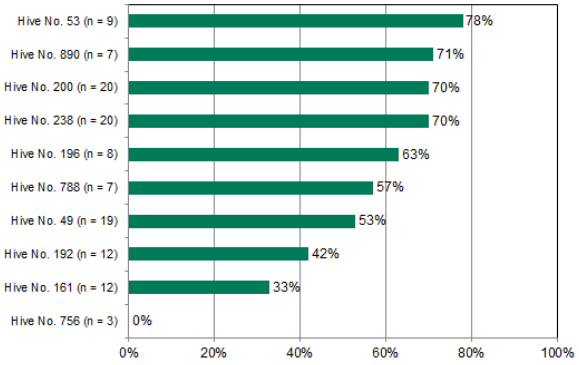


Fig.. 4: Comparison of different larval origins:
% plates with an emergence rate > 70 % on day 22

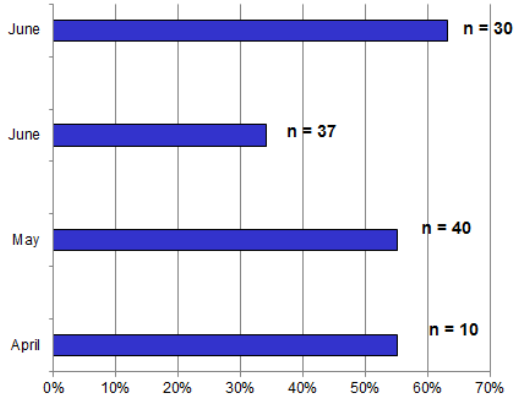


Fig. 5: Comparison of different test periods:
% plates with an emergence rate > 70 % on day 22

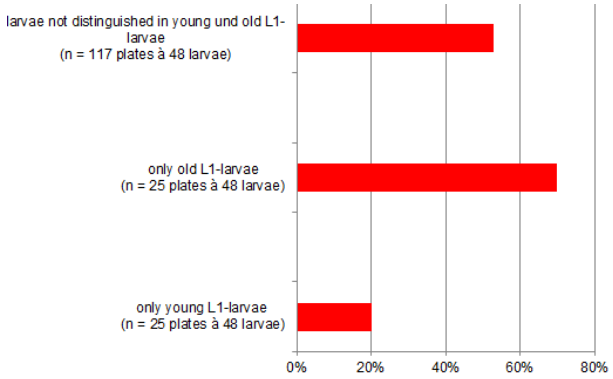


Fig. 6 Comparison of larval age:
% plates with an emergence rate > 70 % on day 22

Conclusions

- For brood tests (laboratory/semi-field/field) which are conducted before summer solstice statistically reliable results can be expected.

- The definition of the BFDs for brood tests (semi-field/field) should be adapted.

- Carrying out the larval lab test with older L1-larvae could allow to fulfil the validity criteria emergence rate > 70% even for fully-chronic feeding the larvae immediately after grafting.