In several semi-field trials according to EPPO guideline No. 170 we compared two different digital monitoring systems (ApiSCAN® and Arnia™ remote hive monitoring) and related the sensor-derived data with usual manual assessments. Based on our findings we want to highlight benefits and limitations of a digital beehive in context of the assessment of potential side-effects of plant protection products on pollinators.

3.3.P Bee colony assessments with the Liebefeld method: How do individual beekeepers influence results and are photo assessments an option to reduce variability?

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Abstract

Colony strength, food storage and brood development are a fundamental part of each honeybee field study. Colony assessments are used to compare and assess those for beehive over time. At present, most colony assessments are made by experienced beekeepers according to Liebefeld method. This method is based on an estimation of areas covered by honeybees, food and brood stages on each side of a comb. Areas are counted from a grid separating the comb side into 8 sections which are protocolled with an accuracy of 0.5 sections. An assessment for a hive takes up to 20 min and even with two field locations, it is necessary to split assessments between beekeepers.

So, it is important to make estimates as comparable as possible. For this purpose, beekeepers practice the assessments on pre-determined photographs to "calibrate themselves". The advantage of the Liebefeld assessment is that the condition of bee hive is estimated with minimum disturbance of the bees. Digital photography is under discussion to gain data with high precision and accuracy with one major disadvantage. To be able to see food and brood stages in photographs, bees have to be removed from combs. This, however, results in a disturbance of the colony – especially if the assessments take place in short time intervals of 7 ± 1 days.

An experiment was performed to evaluate the variation between individual beekeepers and to compare the results to data generated with photographs. For the experiment, five colonies were assessed each by four beekeepers independently according to Liebefeld method. Each comb side of the five colonies was photographed with and without honeybees sitting on it for precise analysis at the computer for a number of bees, nectar cells, pollen cells, eggs, open brood and capped brood. The number of bees and cells with the different contents were generated by an area-based assessment in ImageJ as well as a detailed counting with help of HiveAnalyzer® Software. Data from beekeeper estimations were then compared with assessments based on digital photography. With the results of the experiment, we tried to answer several questions. With the study, we wanted to determine the level of variation between the beekeepers for the live stages and food stores estimated.

Honeybee: Colony assessment; Liebefeld method; digital photography; HiveAnalyzer®

Introduction

In 1983 Gerig introduced a method to assess strength, brood and food of a honeybee colony using a pattern of 8 square decimeters (with ½ square being smallest recorded unit) to assess the content of cells and the number of honeybees on a single comb side.

Our intention was to compare this method in terms of accuracy and precision against methods using weighing and photographs as digital photography offers new technical options that were not available when Imdorf *et al.* (1987) did their study on the reliability of Liebefelder method for honeybee colony assessment.

Improvements and key points need to be taken into consideration to compare the methods such as health of colonies and assessments workload.

Materials and Methods

Each comb of 5 honeybee colonies was assessed by four beekeepers. Three of the bee keepers were experienced, while the fourth started his first season.

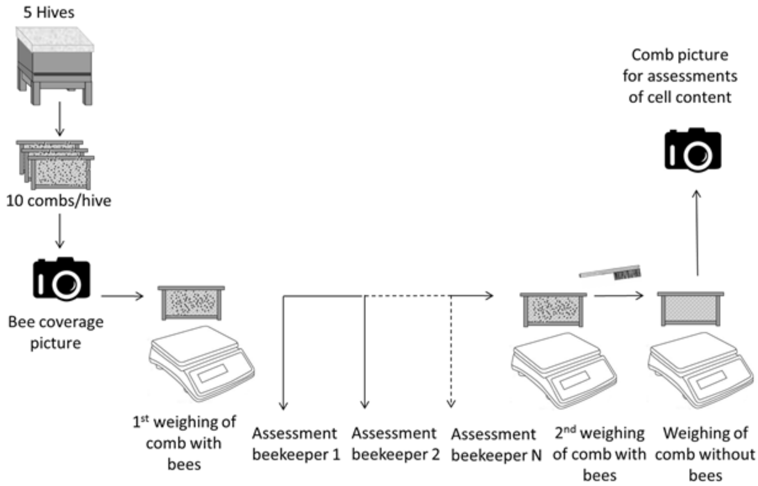

Fig. 1: Order of assessments

At the beginning of the assessment each comb side was photographed with the honeybees. In the next step the frame was weighted. Beekeepers estimated the number of bees, the quantity of brood (eggs and larvae) and the amount of food (nectar and pollen). A second set of photos was taken after the beekeepers finished their assessments. For the photo honeybees were brushed off the comb to count the number of cells with help of HiveAnalyzer© software. The empty combs were weighted without bees (see Fig. 1). On the first set of photos honeybees were counted individually at the computer. Additionally, continuous areas with honeybees were marked on the photos using ImageJ. Numbers of honeybees were calculated from these areas.

As a further method the weight of the honeybees was calculated by the weight difference between a full and empty frame.

The resulting data were compared with each other to receive an estimate about the accuracy and precision of Liebefeld method. The field part of the study was conducted at 1st of April 2019.

Results

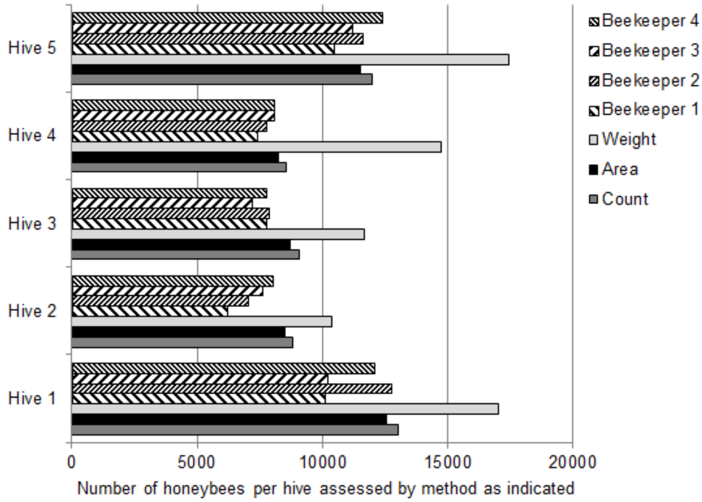

Fig. 2: Assessments of number of honeybees of 5 colonies done by 4 beekeepers and by means of assessment of honeybee weight, measurement of the area of honeybee coverage and direct count of honeybees from digital photos

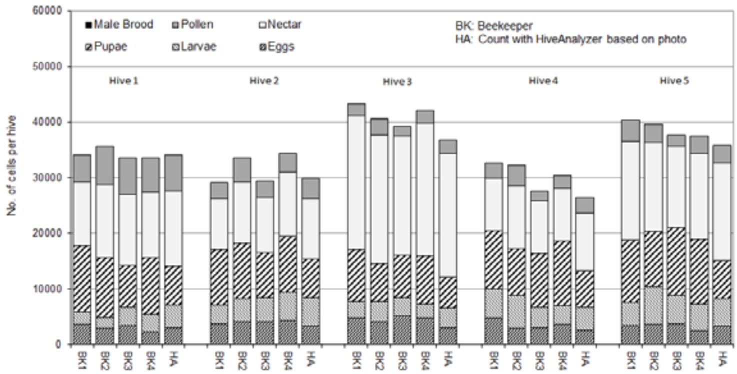

Fig. 3: Assessments of number of brood and food cells of 5 colonies done by 4 beekeepers using Liebefeld method and count from digital photo

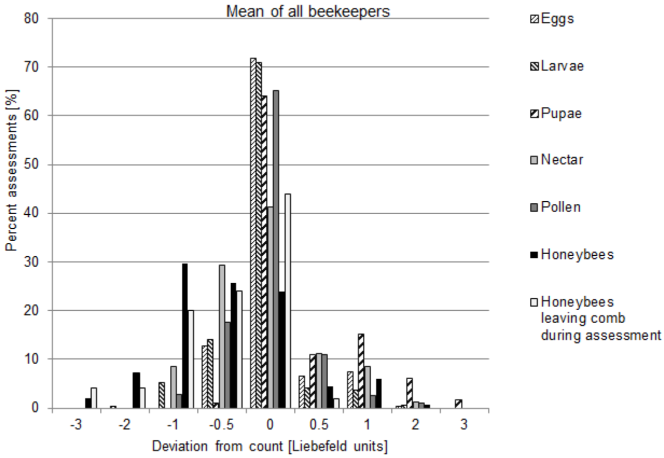

Fig. 4: Mean deviation of beekeeper assessment from count methods and number of honeybees leaving combs during assessment by means of calculation from weight before start of first beekeeper assessment and after last beekeeper assessment

Number of Honeybees

Liebefeld method honeybee estimates were as expected similar between beekeepers. There was no consistent bias between estimates of individual beekeepers. Neither was a beekeeper estimating consistently higher values nor lower values if compared to the average number of bees. Generally, estimates of beekeepers were lower than results from digital photography either as counts or estimation of area covered with bees. The assessment of number of honeybees from the weight of bee mass was not close to the values of the other methods assessed. Weight overestimated the number of bees by a wide margin (Fig. 2).

While Liebefeld estimate takes less than 1 minute per frame counting of bees took 17 minutes per frame side and determination of number of honeybees from area measurement took 30 seconds per frame side.

Precision of beekeeper assessments of honeybees was high. This is indicated by a low coefficient of variation (<12%, Tab. 1). The total number of bees was underrated by up to 21.2%. The deviation was linked to the number of bees indicated by Pearsons correlation coefficient between 0.91 and 0.95 (Tab. 2). The results show that bees in larger hives are more likely to be underestimated.

Weight seems to be not a good way to estimate bee numbers. Weight of each single honeybee can be very variable. Up to 100% can be found if individual bees are weight taken randomly out of the hive. One reason might be their ability to store food in the stomach. This can make up to the half weight of the honeybee.

Tab. 1 Coefficient of variance (cv) of determination of cell contents by beekeepers of each hive and deviation of mean numbers of cell contents and honeybees from counts (n=5)
Absolute values larger 20% marked in bold letters.

	CV [%] by hive 1-5	% deviation from count by hive 1-5
Egg	19.8 / 5.8 / 8.3 / **24.0** / 17.8	-1.1 / **26.6** / **56.1** / **36.6** / -1.8
Larvae	**24.2** / 16.4 / 13.2 / **27.0** / **21.3**	**-33.4** / -18.4 / -11.7 / 10.9 / 5.8
Pupae	17.3 / 9.7 / 13.8 / 13.3 / 8.7	**58.1** / **44.4** / **47.2** / **56.5** / **61.2**
Nectar	6.4 / 9.9 / 5.4 / 8.8 / 8.5	-9.4 / -5.1 / 4.2 / -4.4 / -9.2
Pollen	14.6 / 19.8 / **21.3** / **33.5** / **23.2**	-5.8 / -4.3 / -12.1 / -7.7 / -3.6
Honeybees	11.8 / 11.0 / 3.9 / 4.1 / 7.1	-16.6 / **-21.2** / -18.6 / -11.6 / -8.1

Tab. 2 Coefficient of correlation (Pearsons) and slope of regression line for each assessed parameter for correlation between each comb side assessed by a beekeeper and the count from photo (n= 100). Pearson correlation coefficients lower than 0.90 marked in bold letters.

	Pearsons correlation coefficient by beekeeper 1-4	Slope by beekeeper 1-4
Egg	**0.85** / 0.90 / 0.92 / 0.90	1.47 / 1.40 / 1.47 / 1.22
Larvae	**0.80** / **0.78** / **0.88** / 0.90	0.82 / 1.07 / 0.96 / 1.02
Pupae	0.98 / 0.97 / 0.97 / 0.97	1.66 / 1.36 / 1.46 / 1.55
Nectar	0.97 / 0.97 / 0.94 / 0.97	1.14 / 1.08 / 1.06 / 1.19
Pollen	**0.87** / **0.86** / **0.81** / **0.87**	1.02 / 1.15 / 0.99 / 1.07
Honeybees	0.93 / 0.94 / 0.91 / 0.95	0.88 / 0.94 / 0.89 / 0.98

Number of brood and food cells

The number of eggs, open brood, capped brood (pupae), nectar and pollen was comparable between beekeeper estimates. There was no trend for the estimates being higher or lower for individual beekeepers. One trend was found for the number of capped cells that was always estimated higher by the beekeepers when compared to the photographic method (Fig. 3 and 4). The reason may be the number of empty cells within the field of capped cells. This would lead to an overestimation, if the whole area is taken.

It took approximately 20 minutes to identify each cell content on a comb site with help of HiveAnalyzer© software making it a non-recommendable method for general use.

Fig. 4 shows the mean deviations pattern from counts by beekeepers in terms of Liebefeld units. Coefficient of variation of beekeeper assessments being below 30% (except of pollen assessments with 33.5%) is within an acceptable range of estimation (Tab. 1). Relation of numbers assessed by beekeepers and counts can be described by regression lines with high coefficient of correlation according Pearson (Tab. 2). Slope of these lines could be used as correction factors for parameters where deviation between counts and beekeeper assessments are high. Compared to parameters appearing in large amounts (pupae, nectar, honeybees), correlation coefficient was lower for eggs, larvae and pollen.

Conclusions

Assessments of honeybee colonies according Liebefeld method are reliable and reproducible. Beekeepers introduce almost no individual bias in the estimation.

Assessments of honeybee colonies according Liebefeld method could be adjusted to increase accuracy for an individual beekeeper.

To avoid impact of bias of single beekeepers from statistical evaluation, beekeepers should be distributed over treatment groups (*e.g.*, assessing certain replicate numbers) if several beekeepers work within one study.

Cell contents that appear in lower number like eggs, larvae or cells with pollen are assessed with more variation between the beekeepers (indicated by higher coefficient of variation (CV)) and lower correlation between numbers assessed by the beekeepers and counts from photos (indicated by lower Pearsons correlation coefficients).

For the assessment of capped brood (pupae) cells the beekeepers did their assessment with low variation between each other and high correlation to the counted numbers. But there was a general overrating of capped brood cells by the beekeepers. As this is a stable trend it does not harm the informative value of honeybee studies but it may become troublesome for modeling of hive development and would have to correct for the overestimating done by the beekeepers.

Smaller hives increase the precision of the total estimate.

Assessments of number of honeybees from area on photos are a method comparable to counting individual honey bees.

Impact of weather conditions on the number of forager bees can be reduced by assessing replicate number by replicate number and not treatments as blocks after each other.

Determination of number of honeybees using weighing methods results in an overestimation of honeybees (load of nectar in honey stomach).

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3.4.P Practical and regulatory experience in the conduct of bee residue trials

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Abstract

To ensure the safe use of agrochemicals, today's regulatory system requires an assessment of the environmental risk to bees, as well as an assessment of the dietary risk to humans following the consumption of honey and other bee products. Field trials can provide valuable data to assess the potential exposure of foraging honey bees to agrochemical residues and hence the potential for residues to reach honey consumed by humans.

Introduction

Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey (SANTE/11956/2016 rev. 9) were finalised in September 2018. These guidelines should be implemented by 1st January 2020 to fulfil EU data requirements concerning the placing of plant protection products on the market (Regulation (EU) No. 283/2013, Annex 6.10). Different study types are suggested in the guidelines, with the appropriate study type to be conducted dependent on the active substance mode of action, intended use and available data.

Furthermore, residue trials can provide valuable data to assess the potential environmental exposure of bees as part of the ecotoxicological risk assessment of bees to plant protection products (to be assessed under Annex 8.3.1 of Regulation (EU) No. 283/2013).

For the past several years, Staphyt's field team has conducted experimental GLP field and tunnel residue trials, testing different methods for the collection of various apicultural matrices for