Brood day by day
What can we learn?

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As keepers of honey bees, we all memorise key dates of the development stages from egg to adult for workers, queens and drones, and use these in our day to day examinations of colonies. It is a form of detective work which enables us to work out the recent history and what will happen to our colonies. This simple arithmetic is even more critical in queen-rearing operations: three days an egg, five days a larva, and so on. We all have a grasp, more or less, of these figures, and we are fortunate in that in the honey bee colony, these dates tick by, not exactly without variation, but for the most part like a metronome (unlike bumble bees and solitary bees, where the development periods are much more variable, depending on temperature and food resources.)

There has always been interest in following the lives of individual bees, from egg to emerging adult. For years a common method was to pin a sheet of acetate over a frame of brood, and mark on the acetate the locations of young bees and, with a colour code, the stage of development. This would be repeated over, say, one or two brood cycles (21 to 42 days). The drawback was that it was messy, time-consuming, required accuracy in aligning the acetates, and was disruptive of the colony. In addition, the amount of data that could be handled this way was modest.

In the growing field of ecotoxicology – the study of impacts of toxic chemicals on organisms – there has been a need for more thorough assessments of brood development. This is driven to a large extent by the process of regulatory approval for plant protection products, ie pesticides. In these circumstances, the regulators require comprehensive data on adult and juvenile bee mortality when exposed to field realistic doses. In addition, sublethal effects must be assessed. To capture subtle effects, rigorous and sensitive assessments are necessary.

European and North American regulators favour the use of photographs, particularly for brood data, as they are permanent records which can be verified and are not subject to observer bias in the field. The big leap forward has been the rapid adoption of image recognition technology which can handle massive amounts of data quickly.

The following pages illustrate the techniques used for the PoshBee project, which aims to provide the first pan-European quantification of the exposure hazard of chemicals not only to managed honey bees but also to wild bees, and to determine how chemicals alone, in mixtures, and in combination with pathogens and nutrition, affect bee health.[1]

The key elements to a study[2] are:

1. The camera is digital single-lens reflex with or without a mirror, preferably with 24MP (megapixels) or more.
2. The lens is dependent on the frame size. The images shown here were taken with an 85mm fixed-focal-length macro with autofocus and automatic light metering.
3. Illumination is the key to successfully capturing images of eggs, and perhaps more difficult, first instar (developmental stage) larvae, particularly on frames containing both sealed brood and open brood. Different approaches can provide satisfactory results. In this case illumination was by LED floodlights.
4. The equipment is securely fixed in a mobile unit with wheels, which can be easily moved round the study site by one person. The study frame snaps into a holder. The camera and lamp settings are preselected, making the apparatus simple for field technicians on site. The camera housing is weatherproof so the equipment can be used in poor conditions.
5. Image recognition systems are hungry in both computer memory and processing power. A modest gaming machine with good graphics capacity may be adequate. Several systems are available[3], with multiple optional add-ons depending on needs.

Operating the image recognition system

In each study hive, one frame is selected. The queen is caged on that frame for 24 hours in order to obtain many eggs of similar age. The same frame is photographed repeatedly according to a timetable. These photos are transferred to the computer and identified according to hive and date. The five photos shown here are of the same frame at different times.

The software ensures that the photos match. It then overlays the photos so that every cell registers on every photo – ie, it identifies each cell with a unique identifier. The next step is to identify the contents of each cell, everything from egg to pupa, with pollen, nectar, empty, etc – even rubbish. This is done automatically.

Once this data set is in place, the system looks at every cell which contained an egg in the first photo. It follows the development of each of these eggs on every subsequent photo. If the egg hatches and goes through every development stage, emerging as an adult before the last photo, then that egg is classified as successful. If the egg does not develop to adulthood, ie, it fails at any stage, then that is classified as mortality.

Since the system can easily process data on 500 or even 1,000 individual bees in each hive, it enables a sensitive and reliable indicator of damage to brood. The method is of course more complicated than these brief notes cover, and new technology (such as video image analysis) is being adopted. Nevertheless, it is hoped that this illustration is a useful introduction to the significance and methods of ecotoxicology.

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1 This project receives funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773921.

2 Organisation for Economic Cooperation and Development Guidance Document no. 75 Guidance Document on the Honey Bee (Apis Mellifera L.) Brood test Under Semi-field Conditions

3 For example, Visionalytics hiveanalyzer.visionalytics.de

Photos: Matthew Allan

Brood watching

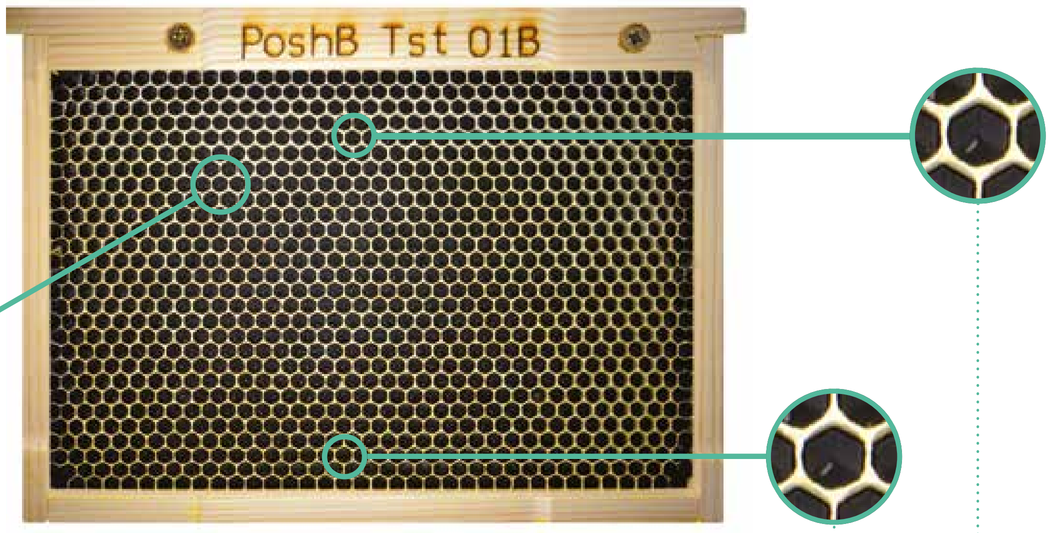
The photographs below show various stages of brood development. It should be borne in mind that it is critical that the contents of almost every cell are visible. This is the case on a reasonably high-quality computer monitor. However, it is not possible to print and see this level of detail on a magazine page.

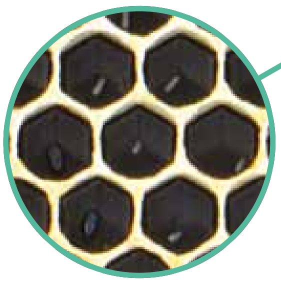
For each photo, a segment of seven cells is enlarged to illustrate points of interest. On the first photo two individual cells are selected; these are enlarged to show an egg in each. On the subsequent photographs, it can be seen that one egg did not reach maturity and one did. This is the type of analysis that the image-recognition software performs, giving a set of tabulated results for each colony in a few seconds.

The first photo in the sequence was taken just as the first eggs laid were hatching, ie between days 3 and 4. Apart from the first 24 hours when the queen was caged, there was no restriction on where the queen could lay.

DAY 1

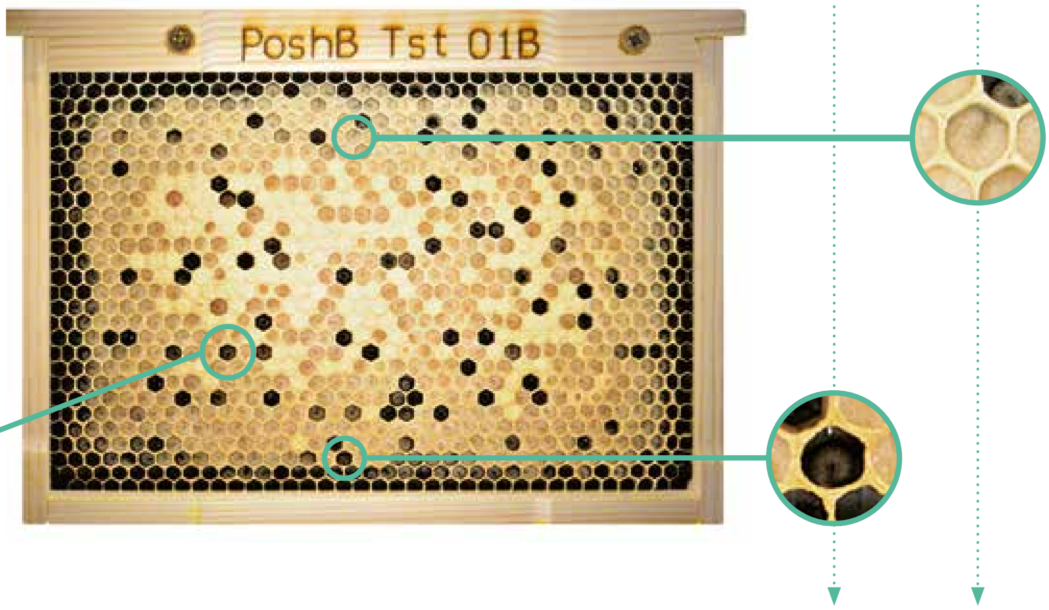
This frame highlights three day-old eggs and a few newly hatched first instar visible in minute quantities of food. The highlighted cells each contain an egg.

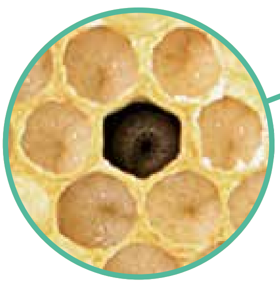




DAY 5

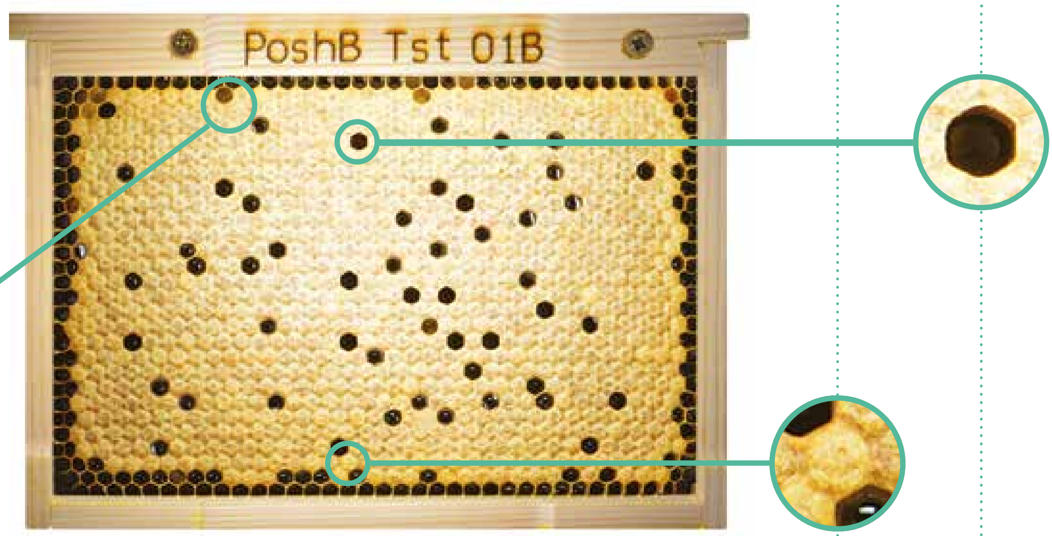
This frame is almost full of old larvae which are rapidly being capped. The enlargement shows a young larva surrounded by older larvae. The dark colour of the larva in the central cell is due to the black plastic foundation used in the special study frames, not to sickness.

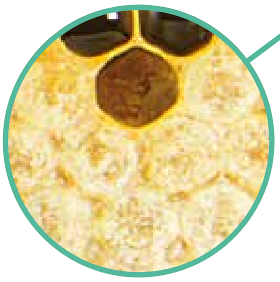




DAY 11

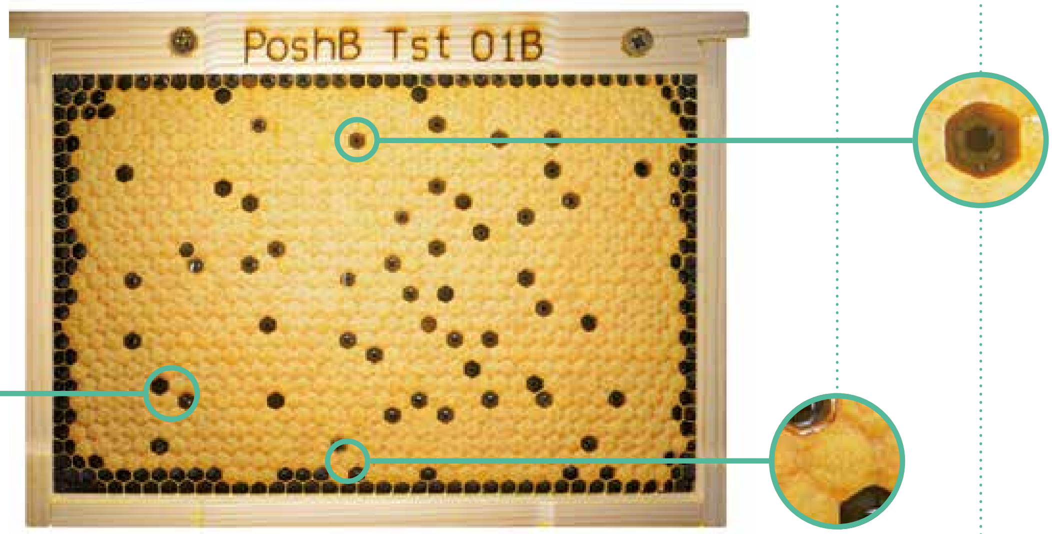
Almost all cells are occupied by pupae under cell cappings. The enlargement shows one cell of pollen surrounded by sealed brood.





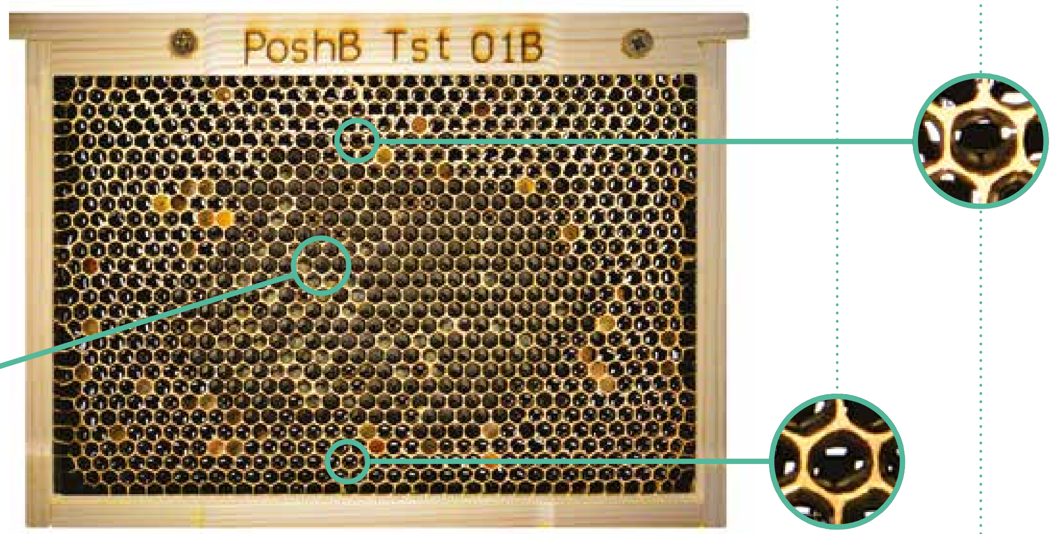
DAY 16

Once the brood is sealed, it is resilient. No mortality has occurred since sealing. The enlargement shows empty cells being filled by nectar



DAY 22

All the old brood has emerged, and the queen has returned to lay in empty cells. The enlargement shows eggs and young brood.



This bee successfully developed from egg to emerged adult

This larva died at a late stage of development