4.4 Technical Innovations In Bumble Bee Semi-Field and Field Tests

Matthew Allan

Atlantic Pollination Ltd, Lendrick, Main Street, Crook of Devon, Kinross, UK, KY13 8AS

DOI 10.5073/jka.2018.462.043

Assessments of impacts on bumble bee colonies in semi-field and field studies rely heavily on technician observation (for example flight behaviour, foraging behaviour) and intervention within the nest (for example determination of production of sexual individuals and quantities of queens reared). The data are time-consuming to collect and present only snapshots. In addition, we assume that manipulation of the colonies and confinement of queens will have an impact on colony development and maintenance.

This presentation comprises a brief overview of some of the technologies that are available to researchers in the field, and a description of a current project which is targeted at improving bumble bee studies by novel application of technology.

Workers in the lab are accustomed to constant improvements in analytical techniques and machinery. In the field however, there have not really been parallel improvements in methods and apparatus. The driving force for this project is the desire for better data, meaning more reliable, more objective and more verifiable data. Associated with these benefits is hopefully an increase in simplicity in field procedures, which enables more work to be done better with more efficient use of staff.

The key factors enabling these objectives are:- (a) cheap and powerful computer capacity, (b) availability of a wide range of sensors, (c) simple programming methods, (d) improved battery technology, and (e) mobile phone technologies. The wide availability of innovative products provides an opportunity to use equipment for uses other than the design purpose. For example, the apparatus shown in Figure 1 was designed by Klostermeyer[1] in 1973 for measuring the weights of individual bees. This was complex, delicate and expensive.

VOL. 46, NO. 4, OCTOBER, 1973 537

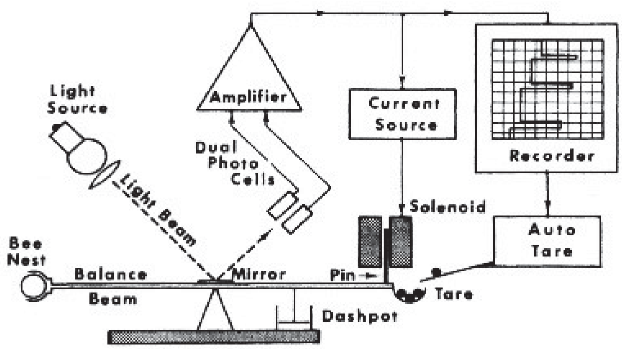


FIG. 1. Diagram of electronic recording balance for obtaining a continuous record of weight changes in nests of *Megachile rotundata*.

Figure 1

Nowadays it is possible to buy scales which are accurate to 1mg, tough, available off the shelf by the thousand and very cheap. Such high-quality equipment sells at such low cost because these scales are essential parts of drug dealers kit; bee researchers can benefit from such 'technology transfer'.

A brief review of technologies currently available for bee researches includes:-

1. **Bee Counters** have been available for years, mostly for honeybee colonies, where the entrance/exit to the hive is split into a number of passages, and the movements of individual bees detected, usually by the bee breaking a beam of light. Evans[2] describes bee counters as part of an integrated honeybee monitoring system.
2. **Image Recognition** – computing power allows large amounts of data to be handled, enabling many images to be scanned and patterns detected.[4] This technology will be familiar to ecotoxicologists as a tool useful for OECD75 studies[5] , where the computer identifies brood stages automatically. (Figure 2) One feature of these systems is the ability to learn, i.e. the more work that is carried out, the more accurate the system becomes. Such techniques can be used for many purposes, such as identifying pollens or insect species.

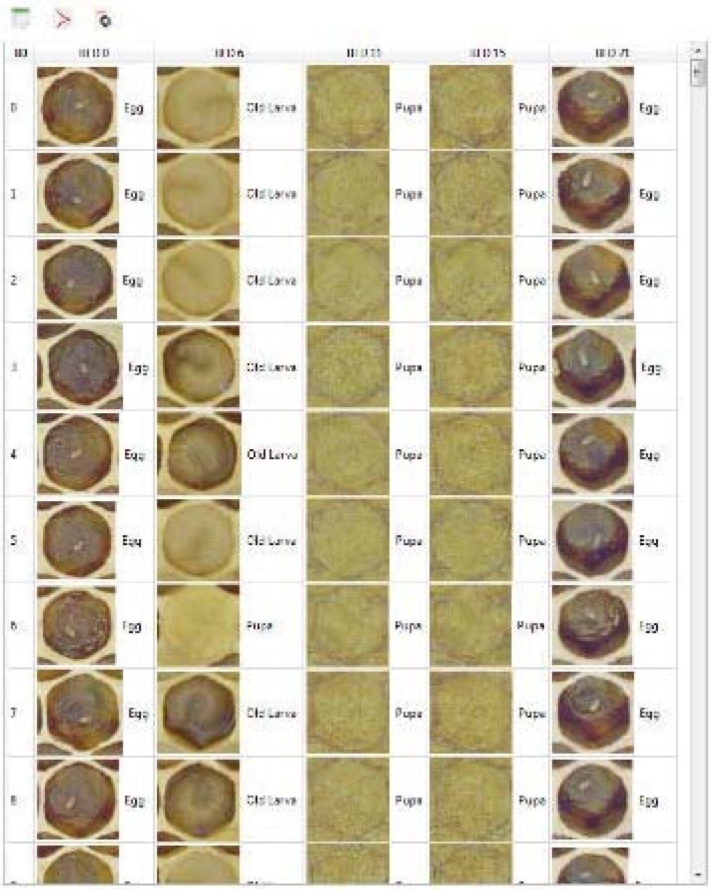


Figure 2

3. **Camera Technology** – image recognition software depends on high quality images. Taking one example, photographing honeybee brood – when taking a photo of a whole frame, it can be challenging to get even illumination of different areas of comb, such as eggs on new wax, and first instar larvae in dark comb surrounded by sealed cells. (Figure 3) A feature common on smart phone cameras (but not so common yet on digital slr's) is HDR (high dynamic range). This in essence captures three or more images of the same shot, and merges them in the camera computer, so that every part of the picture has the optimal exposure. An incidental benefit of digital imaging is the ability to push the

effective film speed to very high levels before breakdown of the image, permitting significant depths of field (particularly advantageous when photographing eggs and young larvae).

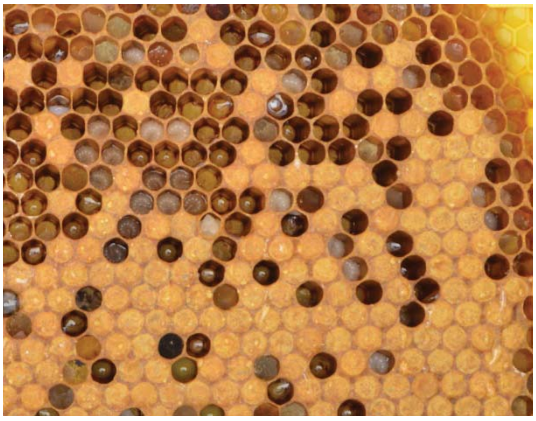


Figure 3

4. **Bee Tracking In Flight** – bees can be tracked in flight using harmonic radar. (Figure 5) The complexities of the system, however, make it less suitable for ecotoxicology than pure research.
5. **Individual Bee Identification** - there are two approaches, both of which require tags to be attached to individual bees – these are radio frequency identification (rfid) tags (which are read by a short-distance scanner at for example a hive entrance)[6] and visual pattern tags which are read by an image recognition system; the latter is probably the more flexible system. Both methods identify a bee at a location at a time, but do not track bee flight.
6. **Laser Bee Tracking** – an exotic technology tested for military use. Honeybees trained to associate food with explosives were tracked by scanning laser which detected wing beats by interferometry. This is a tool with no obvious current ecotox application, but may have use in future.

Whereas these examples range from mundane to science fiction, they underline the fact that technology advances and cheapness are providing the opportunity to take new and innovative approaches to our work. Not only that, but they also open up the possibilities of new and important endpoints.

Novel Apparatus for Bumble Bee Monitoring

The current project is an attempt to improve bumble bee studies both in the semi-field case and the open field. Endpoints conventionally assessed in such cases would include production of young queens; size of young queens; mortality; and flight behaviour.[3]

Examining these in turn: -

Production of young queens is measured by restricting the exit from the nest so that no young queens can leave. This prevents the new queens from carrying out their normal behaviour of leaving the nest to forage and mate. It may be argued that the presence of numerous young queens restricted to the nest, and the associated levels of pheromone influence the colony behaviour, thereby creating a situation which is far from the ecotox ideal of being as 'natural' as possible. At a series of inspections each young queen is manually removed by a technician, a time-consuming and awkward procedure in the field.

Mortality within the nest box is assessed by opening each nest periodically and counting and removing dead bees, adults and juvenile. There is no current method of counting dead bees that are cleaned out of the colony by workers, because the variation in body size precludes the use of dead bee traps as used for honeybee studies. There is no current method of getting absolute counts of forager mortality, although comparative counts can be made of dead foragers on fabric laid in areas cleared of crop.

Flight behaviour is conventionally measured by observation, typically by a technician watching a nest for ten minutes or more. Relatively low numbers of flights are recorded, and the observation process may be spread over several hours, with the possibility of variability due to changing weather conditions, light levels, presentation of nectar and pollen, and the practice of assessing all control enclosures before the treatment enclosures.

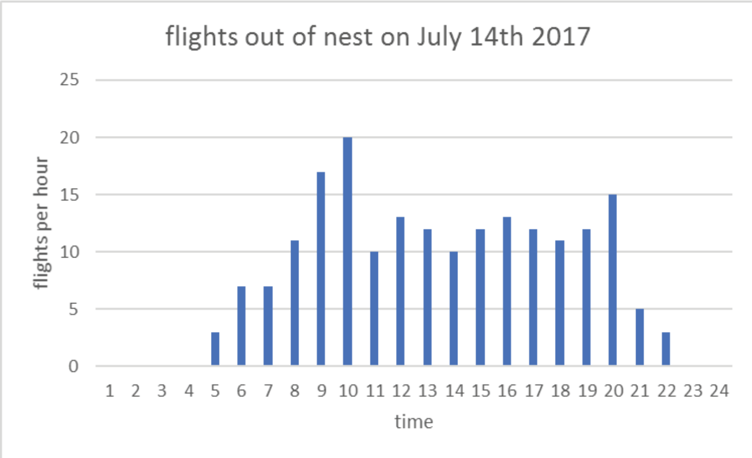
Figure 4

To develop alternative approaches to the above, six electronic bumble bee monitoring units (Figure 4) were built in 2017. They were placed in a field of phacelia adjacent to a semi-field bumble bee ring test in order to compare novel and conventional methods. Each comprised a cabinet with two compartments – one for the bumble bee nest (a commercial pollination nest), and an adjacent compartment into which different technical units can be fitted. The technical units can be built with different sensors and other functions and simply drop into the cabinet. The basic functions are (a) detection of every bee entering the nest, (b) detection of every bee leaving the nest, and (c) photographing every bee entering or leaving the nest. (It should be noted that the system does not require individual bees to be tagged.)

A key element is the arrangement of passages through which the bees enter and leave. Early trials used flaps or gates to ensure one-way traffic only. However these were rejected as they tend to cause congestion and false readings. A better approach was an arrangement of passages which present large openings and small unobtrusive exits. These have been very successful, particularly in the case of bees entering the nest; returning foragers fly into the funnel and quickly and unhesitatingly go through the passage into the nest. The clear passages (acrylic or glass) are arranged to run side by side so that one camera 'sees' both. In use there has been no problem of blockage with dead bees or debris. Sensors built into the passages detect movement and record date, time (hours, minutes, seconds) and direction of movement. A miniature camera system (still in development) records an image and links it to the time log.

The data is stored on a micro-SD card which can be read at any time during the study or removed at the end of the study. The electronics are designed and built to operate on very small power demand so that the unit can be left in the field for the duration of the study without changing or re-charging batteries.

The units were placed in a field of phacelia adjacent to the cages of a semi-field bumble bee ring test which was being carried out in a conventional manner, in order to obtain some comparisons. Typically over a three week period, the data recorded included approximately 4,000 bees leaving the nest and a slightly lower number entering the nest. The data is downloadable to an Excel file where it can be easily manipulated as required. For example flights per day can be examined or flights per hour through the day. (Figure 5)



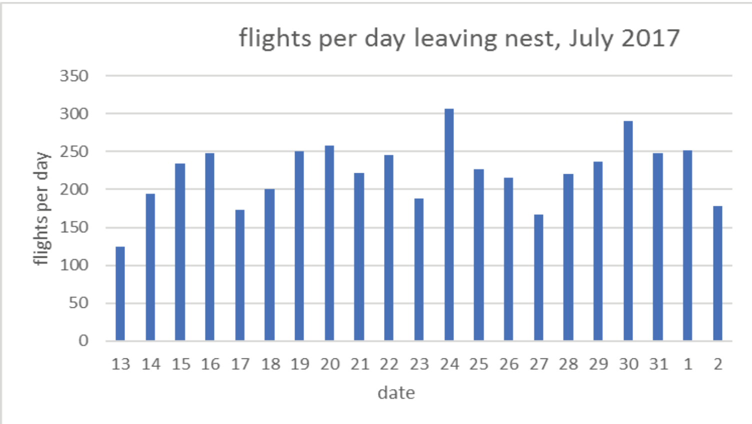


Figure 5

Data from a weather station on site can be flowed into the same spreadsheets.

Comparing these data with those from the adjacent ring test, there are 4,000 data points collected using the electronic monitoring unit, compared with, typically, less than 30 data points recorded by field technician. While large data sets are not necessarily an improvement, in this case the data sets can be handled to give a clearer picture of impacts, and more sensitive assessment methods.

By recording and comparing the inward movements and the outward movements of the bees, the number of bees that leave the colony and do not return can be calculated. This makes possible a new and significant end point – the mortality of foragers. This endpoint is also of importance in open field studies.

The flight activity of workers undergoes a sharp decline at or near the switch point. Although the method has not been tested yet, it is probable that the extensive data set of flights could be used to pinpoint the timing of the switch point.

The other function incorporated in the monitoring units was the photographing of every bee movement. This was to detect queens which were allowed to fly freely, in contrast to being trapped in the nest by queen excluder. First, however, it was necessary to confirm that queens of the species (*Bombus terrestris* subs. *terrestris*, and *B. terrestris subs. audax*) used in studies in Europe are distinguishable from workers and drones by size alone. These species (and *Bombus impatiens*, which is provided for commercial pollination in North America) are pollen-storers as opposed to pocket-builders. It is regarded as a characteristic of pollen-storers that there is no or little overlap in size between queens and other bees. The chart (Figure 6) shows the size distribution (measured in milligrams) of all the bees collected from six control colonies at the end of a study. It can be seen that the masses of drones and workers are very similar, and that the overlap with queens is almost zero (one small queen can be seen at 350mg.). It therefore is realistic to use a size measurement to differentiate queens with little chance of mistaken identifications.

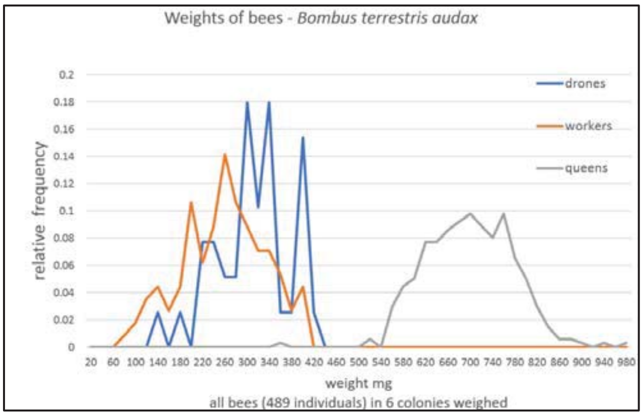


Figure 6

Each photo shows the two parallel passages, in and out, through which all bees pass. When downloaded, they are conveniently viewed as thumbnails which can be scrolled through for identification. (Figure7) Queens at 20mm+ long (at lower left) are easily distinguished from drones and workers at 12 to 14mm long.

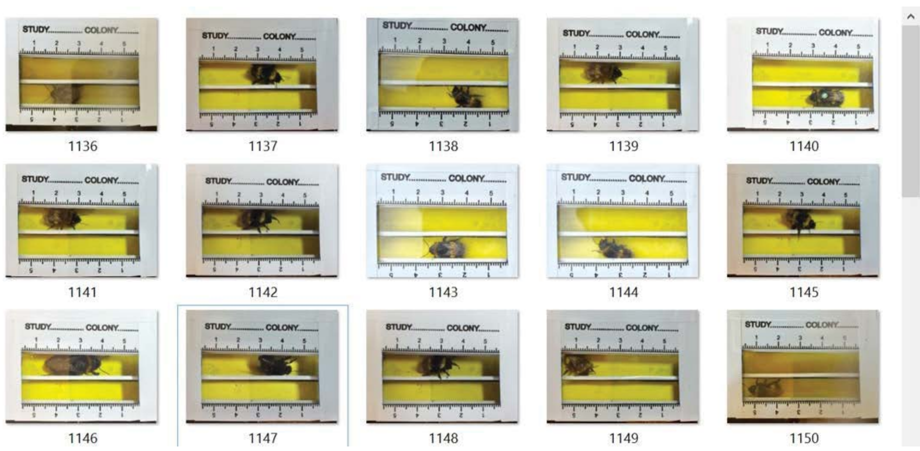


Figure 7

Since the normal behaviour of young queens is to make several return trips to the nest to mate and feed, before finally leaving, it is simple to calculate how many queens leave the nest each day, and hence the total number of young queens produced. This method of assessment is non-invasive, quick, simple and reliable.

The photographic method was developed with a view to writing image recognition software to automatically scan each photo to identify and calculate the number of young queens. Such a system can be programmed to recognise and measure such physical characteristics as thorax diameter, wing length, overall body length and abdomen width. Providing image quality is adequate, it is capable of counting antennal segments to differentiate between workers and drones.

In a final trial of the year an add-on weighing module, using the above drug dealer scales, was used to measure the weights of marked bees entering and leaving, in order to gain information on pollen and nectar inputs. This may assist in assessing exposure as a precursor to developing impact assessments.

Summary

The following functions can potentially be incorporated into the apparatus:-

- Download data by phone, bluetooth or wi-fi.
- Automatically weigh every bee.
- Automatically weigh the nest.
- Photograph pollen loads.
- Differentiate between workers and drones.
- Track individuals by automatic pattern recognition.

The benefits of the apparatus include:-

- Data collection methods are non-intrusive.
- Data recording is automatic.
- More sensitive assessments can be made.
- Staff time in the field is reduced.

Useful new endpoints are possible in both semi-field and field studies, for example:-

1. Forager mortality.
2. Pollen collection.
3. Flight behaviour.
4. Timing of switchpoint.

References

1. Klostermeyer E C, Mech S J, Rasmussen W B (1973): Sex and weight of Megachile rotundata (hymenoptera Megachilidae) progeny associated with provision weights. *Journal of Kansas Entomological Society* **46**: 536-548
2. Evans S K (2014): Electronic beehive monitoring – applications to research. *Hazards of pesticides to bees. 12th International Symposium of the ICP-PR Bee Protection Group 15 to 17 Sept 2014, Ghent. Proceedings;* 121-129
3. EFSA (2014): Guidance on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus spp. and solitary bees*). *EFSA Journal* 2013; 11(7):3295, 266pp., doi:10.2903/j.efsa.2013.3295
4. Hiveanalyzer image recognition system. http://hiveanalyzer.visionalytics.de
5. OECD (2007). Guidance document on the honey bee (*Apis mellifera* L.) brood nest under semi-field conditions (http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2007)22&doclanguage=en)
6. Scheiner R, et al (2013) Standard methods for behavioural studies of *Apis mellifera*. *Journal of Apicultural Research* **52**(4): 31-36

462

Julius-Kühn-Archiv

Pieter A. Oomen, Jens Pistorius (Editors)

Hazards of pesticides to bees

13th International Symposium of the ICP-PR Bee Protection Group

18. - 20. October 2017, València (Spain)

- Proceedings -

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

History ICPPR-Bee Protection Group conferences

1st Symposium, Wageningen, the Netherlands, 1980
2nd Symposium, Hohenheim, Germany, 1982
3rd Symposium, Harpenden, UK, 1985
4th Symposium, Řež, Czech Republic, 1990
5th Symposium, Wageningen, the Netherlands, 1993
6th Symposium, Braunschweig, Germany, 1996
7th Symposium, Avignon, France, 1999
8th Symposium, Bologna, Italy, 2002
9th Symposium, York, UK, 2005
10th Symposium, Bucharest, Romania, 2008
11th Symposium, Wageningen, the Netherlands, 2011
12th Symposium, Ghent, Belgium, 2014
13th Symposium València, Spain, 2017
14th Symposium scheduled, Bern, 2019

Organising committee 13th conference

Dr. Jens Pistorius (Julius Kühn-Institut, Germany)
Dr. Anne Alix (Dow Agrosciences, United Kingdom)
Dr. Carmen Gimeno (Trialcamp, Spain), local organiser
Dr. Gavin Lewis (JSC, United Kingdom)
Dr. Pieter Oomen (Wageningen, The Netherlands)
Dr. Veronique Poulsen (ANSES, France)
Dr. Guy Smagghe (Ghent University, Belgium)
Dr. Thomas Steeger (US Environmental Protection Agency, USA)
Dr. Klaus Wallner (Hohenheim University, Germany)

Editors

Dr. Pieter A. Oomen, Wageningen, The Netherlands
Dr. Jens Pistorius, Braunschweig

Group photo of all symposium participants, standing in front, from left:

Thomas Steeger (new board member),
Jens Pistorius (new chairman),
Françoise & Pieter Oomen with award (editor & former chairman),
Guy Smagghe (organiser, symposium host and new board member),
Job & Margreet van Praagh with award,
Anne Alix (secretary of the board)

Foto

Pieter A. Oomen (Bumble bee *Bombus lapidarius* on thistle)

The proceedings of the symposia (such as these) are being published by the Julius Kühn Archive in Germany since the 2008 symposium in Bucharest, Romania. These proceedings are also accessible on internet, e.g. the former symposium proceedings published by JKI can be found on https://ojs.openagrar.de/index.php/JKA/issue/archive (Issues 423, 437, 450). Furthermore, proceedings of former meetings have meanwhile been digitalized and can be found on https://www.openagrar.de/receive/openagrar_mods_00032635.

Bibliografische Information der Deutschen Nationalbibliothek

Die Deutsche Nationalbibliothek verzeichnet diese Publikation. In der Deutschen Nationalbibliografie: detaillierte bibliografische. Daten sind im Internet über http://dnb.d-nb.de abrufbar.

ISSN 1868-9892
ISBN 978-3-95547-064-7
DOI 10.5073/jka.2018.462.000

Printed in Germany by Arno Brynda GmbH, Berlin.