The Hygienic Behaviour Study

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Interesting results have emerged from the two-year study of hygienic behaviour at the National Bee Unit

The National Bee Unit has carried out a survey into the occurrence of hygienic behaviour in England and Wales over the past two years, with the assistance of both Appointed Bee Inspectors (ABIs) and beekeepers. Hygienic behaviour is often cited as a defence mechanism against both American Foul Brood (AFB) and chalk brood. Adult honey bees showing it detect dead larvae in cells and remove them before bacterial or fungal spores have a chance to develop within diseased larvae, thus limiting spread throughout the colony.

There are several assays for hygienic behaviour. One method often used is the pin-prick method. This involves killing a sealed larva *in situ* using a pin, without interfering too much (if at all) with the cell capping. There are several problems with this method, however. Generally, relatively few cells are used for each assay, which may give misleading results. The larva may not be killed by the treatment, or leakage of cell contents may cause bees to detect this haemolymph rather than dead larvae. Also, if the integrity of the capping is disturbed, bees may remove dead larvae that would not normally have been detected.

FREEZE-KILLED LARVAE

Another technique for testing is to use freeze-killed larvae. There are two main methods, using either liquid nitrogen or freezing in a domestic freezer to kill a section of brood. If liquid nitrogen is used, this can be applied directly onto the brood comb. This method must be carried out very carefully, as liquid nitrogen is extremely cold and spillage may lead to serious injury. If a domestic freezer is used, a section is cut from the comb and frozen for 24 hours precisely. The piece of comb is reinserted into the frame and the number of larvae that have been removed by the bees after 48 hours is counted; hygienic behaviour is indicated by removal of greater than 95% within this time. We decided that using the domestic freezer was most appropriate for the type of survey we had in mind.

2000 AND 2001

In 2000, all the survey participants were ABIs and in 2001, volunteers were recruited via their local beekeeping associations. The experiments were slightly different as, in 2000, ABIs were given the option of freezing whole combs and indicating areas of both sealed and unsealed brood. This eliminated comb cutting and meant removal of unsealed brood could also be assayed. Most assays (23 out of 37) recorded figures for unsealed brood. In 2001, the only method advised was to cut a section of sealed brood from the comb and freeze it.

RESULTS

The results gained over the two years are very interesting and are discussed as two separate years. Thirty-seven colonies were assessed for hygienic behaviour in 2000, with just under two-thirds also being tested for removal of dead, unsealed brood. The method used by ABIs was according to their personal preference and the amount of brood present. As all of the assays were carried out towards the end of the season this latter point was an important consideration. The first observation of note was the removal of dead, **unsealed** brood in 2000. In most cases, all dead brood was removed; in only three cases was less than 95% of brood removed. Although this was not part of the assay for hygienic behaviour, it was interesting to note as this may mean that it is the detection of dead larvae, rather than their removal, which influences expression of the behaviour. Six of the 37 colonies showed hygienic behaviour, with greater than 95% of dead, **sealed** brood removed, giving a level of 16%.

In 2001, 41 colonies were assayed, this time just for removal of dead, **sealed** brood. A lower percentage of colonies showed hygienic behaviour, just two of those tested (5%). Some beekeepers tested the same colony twice by assaying both sides of the comb for removal of larvae so, in total, 53 assays were carried out. Although these results for both sides were generally very similar, there were some where values differed significantly. The most noteworthy of these was a colony where 100% of dead larvae was removed on one side, but just 21% on the opposite face. This is very intriguing and it is not altogether clear why there is this difference. It is possible that one side of the frame was damaged during the freezing process, which led bees to investigate the cells to repair the cappings, resulting in the higher removal rate.

Another notable observation was the range of dead larvae removal. There was not an 'all or nothing' response, but variable amounts of brood were removed, as shown in Fig 1 (illustrating

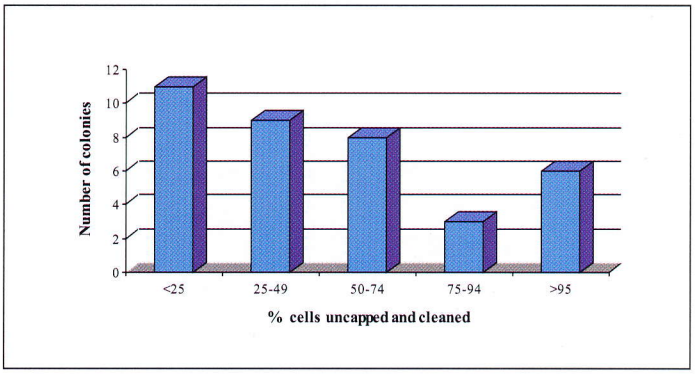

Fig 1. Removal of dead larvae from colonies assessed for hygienic behaviour expression in 2000

Fig 2. Sample cut out from the frame

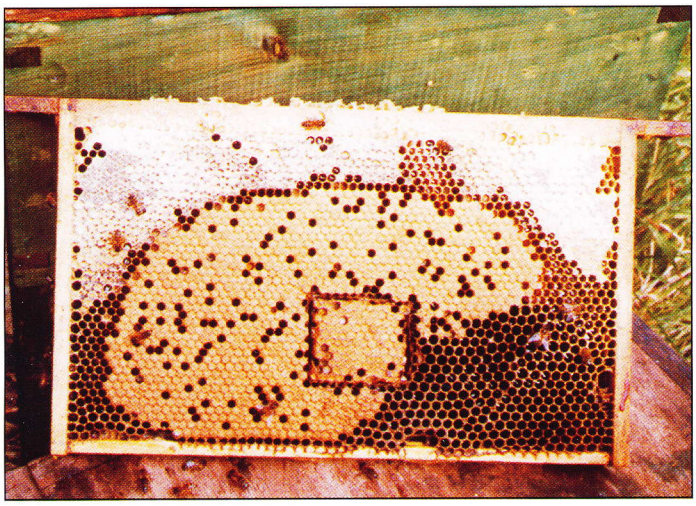

Fig 3. Sample back in place after freezing

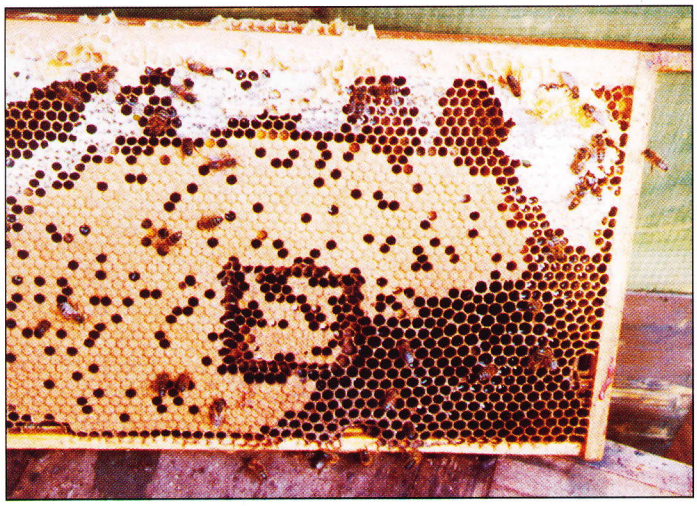

Fig 4. Sample after 48 hours
(Note that comb has been built around the section)

only 2000 data). It is very likely that more dead larvae would have been removed from the sections if they had been allowed to remain in the colonies for a greater time period. Indeed, it has been said that any colony will remove dead larvae if they are left in a colony for long enough, which is entirely logical. However, to show hygienic behaviour for the freeze-killed method used, the time-scale is removal within 48 hours.

SEVERAL INFLUENCING FACTORS

Expression of hygienic behaviour is dependent on several conditions, both within the hive as well as outside, and also on the genetics of the bees. The genetic basis for the behaviour it is not fully understood. Environmental factors, such as the presence of a honey flow, and factors including the ratio of adult bees to brood will also affect expression of the behaviour.

PARTIAL HYGIENIC BEHAVIOUR

What we have found in England and Wales is very interesting. Obviously, some colonies showed hygienic behaviour. The original aim of the experiment was to determine what percentage of colonies show hygienic behaviour and to determine whether or not this could be tied into areas with high levels of disease. From these results, an average of 10.5% of colonies tested expressed hygienic behaviour. No geographical link with disease occurrence was evident, but as the dataset was relatively small, this was not surprising. It is likely that there are many colonies expressing 'partial' hygienic behaviour, where some dead larvae would be removed if tested, but at a level lower than 95%. This is good news from the beekeeping point of view as, if colonies show some hygienic practices such as removal of at least some diseased larvae, this may help to prevent rampant spread of disease within the colony. In the USA, it has been estimated that approximately 10% of colonies express hygienic behaviour, similar to the level found on average over the two years in the current study. Further research into the genetic basis and the mechanisms of detection of dead larvae are currently ongoing in the USA.

TRY IT YOURSELF

Hopefully, beekeepers will be able to follow the methods given here if they wish to determine whether their colonies show hygienic behaviour or not. It is a good idea to carry out the assay at least twice at different times during the season to ensure the behaviour is consistent, especially if the colony is going to be used for breeding purposes specifically for hygienic behaviour.

If you would like further information about the methods used here, or if you have any questions, please contact the NBU, Central Science Laboratory, Sand Hutton, York YO41 1LZ. ✻

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