

Containing the Small Hive Beetle for Research Purposes

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We learn about the NBU containment facilities for the small hive beetle

INCREASED WORLD trade in bees provides opportunities for exotic pests to cross international borders. The small hive beetle (SHB) (*Aethina tumida*) (Figure 1) is indigenous to Africa, where it feeds and breeds inside honey bee colonies, causing comparatively little harm. However, outside its native range, within colonies that lack African bees' natural defences, adult SHB enter hives unchecked, causing devastating infestations.

The beetle has a very high reproductive rate, enabling populations to expand very rapidly under favourable conditions. Larvae feed on pollen and honey, while adults prefer brood. As a result of larval feeding, the honey becomes watery and fermented. Adults feeding on brood can seriously affect the colony's reproductive abilities and, in severe cases, whole colonies can be lost.

The SHB is readily spread through movements of colonies, beekeeping equipment, hive products, soil and fruit. As a result, it now thrives in North America. SHB was first detected in Florida in 1998 and has since spread to 31 US states. In one year, in Florida alone, 30,000 colonies were lost; infestation damage and honey contamination cost the industry \$3 million. Since 2002, SHB has also become established in Australia. It is currently absent from the UK and the European Union (EU), but in 2004 it was intercepted on a consignment of queen bees imported into Portugal from Texas. Although immediate destruction of affected apiaries eradicated the pest, this incident highlights the reality that SHB could arrive again in the EU.

In May 2010, the World Organisation for Animal Health (Office International des Épizooties) received the first report of SHB

Figure 2. The Fera laboratories at Sand Hutton – home of the National Bee Unit



National Bee Unit

Figure 1. The small hive beetle (*Aethina tumida*)

in the US state of Hawaii. This also had serious implications for UK beekeepers. Although the European Commission has since removed the derogation that allowed the import of Hawaiian bees, until that point England and Wales were receiving over 4000 queens from this source each year (80% of the UK's total queen import from outside the EU).

THE RESPONSIBILITIES OF THE NATIONAL BEE UNIT

The key aims of the National Bee Unit (NBU), located in The Food and Environment Research Agency (Fera) (Figure 2), are to protect the honey bee and support our beekeeping industry.

To achieve this, one of our primary roles is to manage the risks associated with the importation of exotic pests and diseases of honey bees, in the event that such species should be introduced into the UK. Because of this, the NBU undertakes practical research projects focused on developing early detection tools for SHB and effective methods for its swift control.

For these reasons it is necessary for us to work with live beetles. Maintaining stocks of any such potentially harmful exotic pest requires highly sophisticated and secure containment facilities. This article describes the state-of-the-art Quarantine Entomology Unit (QEU), housed at Fera's Sand Hutton Laboratories, and explains how it is used to contain SHB safely.

THE QUARANTINE ENTOMOLOGY UNIT

The QEU is a unique facility, which the Department for Environment, Food and Rural Affairs (Defra) cites as being

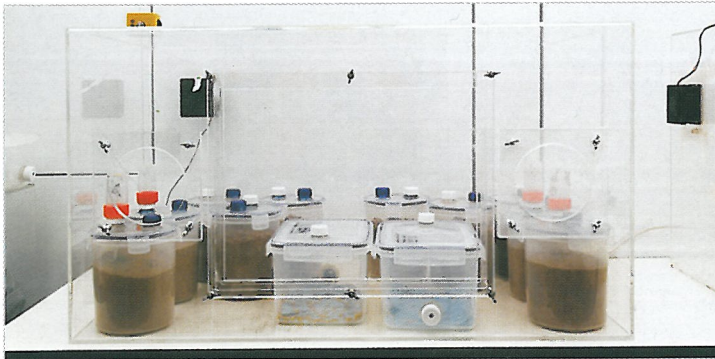


Figure 3. Perspex culture cage with plastic containers holding populations of SHB pupae, buried in sand



Figure 4. The cold corridor of the QEU, maintained at a temperature of $-15\text{ }^{\circ}\text{C}$

its national standard for research into non-indigenous (ie, non-native) pests. Defra and The Welsh Assembly Government (WAG) have issued Fera with a Plant Health Licence (current no. PHL 251D/6211(11/2009)(Amended 08/2010)), that allows us to import, move and keep prohibited invertebrates, including SHB.

Fera's QEU facilities provide such a significant level of containment that this is the only institute within the UK to hold such a Licence for keeping extremely small, potentially high impact pests. In fact, the flexibility of the Licence and the QEU allow Fera to hold any exotic pest species in question, should the need arise. Examples of other high profile species of concern to Plant Health, held under the same Licence, include Asian long-horned beetles, *Anoplophora glabripennis*, and the melon thrips, *Thrips palmi*.

LEVELS OF CONTAINMENT FOR SHB

An integral part of keeping exotic organisms is ensuring that multiple levels of containment are maintained, to keep the risk of escape to an absolute minimum. The highest risk organisms (of which SHB is one) must be kept under at least three levels of containment, but in practice, Fera provides at least two further levels of containment in addition to this.

The levels of containment for SHB are achieved as follows:

Level 1

SHB individuals (eggs, larvae, pupae or adults) are kept within sealed plastic containers (Figure 3).

Level 2

These containers are kept within a purpose-built sealed Perspex culture cage (Figure 3).

Level 3

The culture cages are kept in a sealed controlled environment (CE) room. This has its temperature, humidity and lighting regimes electronically controlled to simulate the environmental conditions preferred by the SHB. The only access to the CE room is via a frozen corridor maintained at $-15\text{ }^{\circ}\text{C}$, a temperature so low that it will rapidly immobilise/kill any insect (Figure 4).

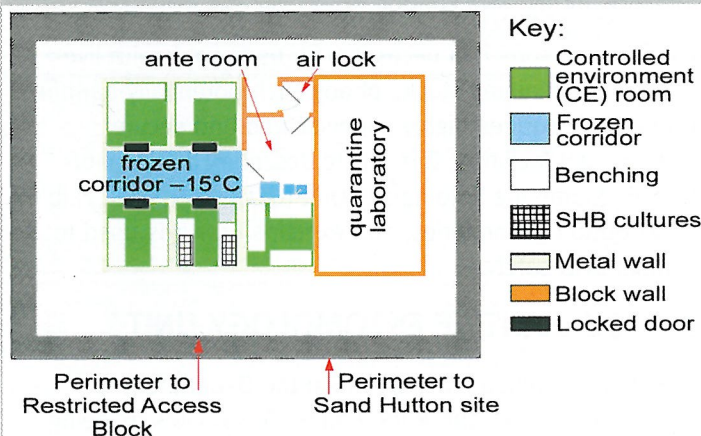
Level 4

The QEU and its integral CE rooms are encased within a sealed metal shell, the entirety of which is maintained under negative pressure: fans pull air out of the QEU through very fine filters, ensuring that no insects can ever escape through the ventilation system. The QEU has no windows and all doors are airtight and held closed under negative air pressure. Waste water is filtered and sterilised within separate quarantine drainage tanks.

Level 5

The encased QEU itself is a self-contained and isolated unit, housed inside another building to which access is restricted, hereafter referred to as the Restricted Access Block (RAB) (Figure 5).

Figure 5. Diagrammatic representation of the Quarantine Entomology Unit facilities at Fera



FACILITY INSPECTIONS AND LICENCE RENEWAL

As a licensed facility, the QEU undergoes rigorous annual inspections, during which a Plant Health Inspector reviews the containment facilities to ensure that the requirements of the Licence are all fully met. There is also a programme of occasional audits of the facilities by independent organisations to review the structural integrity of the unit, the way the facility is maintained and managed, and the procedures and safety measures in place for all the research that goes on within the unit.

It is the responsibility of the Fera Licence holder, who is independent from the NBU and the SHB research team, to keep an overview of the activities within the unit to ensure that the Licence conditions are being adhered to. All the

research activities are the responsibility of Fera's Applied Entomology Team and the lead on the quarantine-based projects, including those involving SHB, is taken by Senior Entomologist, Dr Andrew Cuthbertson.

STAFF ACCESS TO THE SHB STOCKS

Under the terms of the Licence, only a limited number of trained, authorised staff is allowed any access to the SHB stocks and this is strictly for the purposes of culturing the insects, to undertake research experiments, or for training.

The sequence of procedures that must be followed to enable an authorised member of staff to reach the SHB stocks is as follows:

- 1 In the first instance, he/she cannot enter the Fera site at Sand Hutton without initial security clearance.
- 2 Once on site, only staff with additional security clearance can enter the RAB housing the QEU. His/her electronic security pass must be especially encoded to permit entry into this block; access is obtained by swiping the pass over a keypad to the side of the locked door. Staff without additional authorisation will be unable to open this door.
- 3 To enter the QEU itself, the staff member must go through two doors (Figures 5 and 6), separated by a very small hallway that serves as an airlock. The first door to the QEU is locked and the staff member can only enter once even further electronic security clearance has been officially obtained from the Manager of the QEU. As soon as the staff member has passed through this first door, he/she must ensure that it is properly closed behind him/her. Failure to do this before the second door is opened will result in an alarm being activated. Only one door can be open at any one time.
- 4 Upon entering the QEU anteroom, the staff member must put on a laboratory coat. This is coloured red to distinguish it from coats worn on other parts of the site. As a further precaution, between uses, all QEU laboratory coats are stored in a freezer (-18°C).
- 5 The staff member may now enter the frozen (-15°C) corridor, from which the CE room housing the SHB stocks is accessed. The door to this frozen corridor is locked and alarmed; the door to the CE room is also locked and alarmed.
- 6 Immediately upon entry to the CE room, its door must be closed. Should the door to the frozen corridor and the door to the CE room ever be open simultaneously, their alarms will sound continuously.
- 7 The staff member is now in the room that houses the SHB cultures. However, all beetles (eggs, larvae, pupae and adults) are contained within sealed plastic boxes that are themselves contained in larger, sealed Perspex culture cages (Figure 3). Plastic boxes are transparent, as are the Perspex cages, so that the insects are clearly visible at all times.

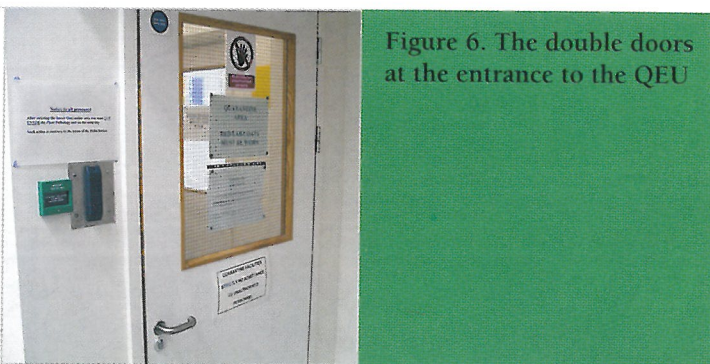


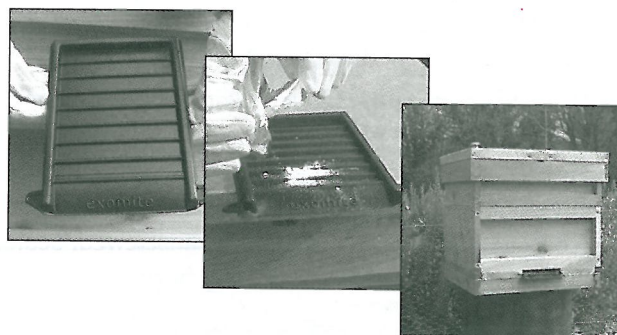
Figure 6. The double doors at the entrance to the QEU

- 8 When insects are 'handled' for the purposes of culturing or research and development, staff members do not work alone, but in pairs. When it is necessary to move insects between containers for culturing purposes, individual insects are literally 'counted in' and 'counted out', ensuring that every specimen is accounted for.
- 9 Staff maintain highly detailed culturing records which log population numbers and development times. This allows us to keep track of all SHB stocks for which we are responsible.
- 10 During the entire culturing process, live insects never come into direct contact with staff; access to the Perspex tanks is through arm-length sleeves that are integral to the tank itself (Figure 7). This design is somewhat similar to that used by scientists working with highly pathogenic microbes.
- 11 All waste materials generated during culturing, including dead insects, are sealed into plastic clinical waste bags

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Figure 7. A scientist at work in the CE room, showing arms inserted into the Perspex culture cage through integral sleeves. Note also the inverted collection cylinders attached to the top of the culture box

and placed into a freezer (-18 °C) for at least 48 hours, prior to disposal by incineration.

- 12 Staff are not allowed to take any equipment, including writing materials, in or out of the QEU unless these have been stored in -18 °C for at least 48 hours.

MAINTAINING THE SHB CULTURES

The SHB cultures held by Fera were originally established from specimens of *A tumida* supplied by the United States Department of Agriculture and the Plant Protection Research Institute, South Africa. These insects were imported under three levels of containment, according to the conditions laid down under the Plant Health Licence, in 2006 and 2007, such that we now hold three different strains: one from SHB's endemic range in South Africa and two from the USA (Maryland and Florida, respectively).

The first population to arrive is now in its seventeenth generation. Cultures are maintained at 20 °C, 65% relative humidity, under a controlled light/dark regime. Adult beetles emerge inside sealed containers of sand in which they pupated.

SHB adults have a natural tendency to move upwards, away from the surface of the sand. Each plastic culture box has a smaller inverted plastic cylinder, baited with a source of honey, attached to its upper surface (Figures 7 and 8). When a number of adult beetles have congregated in one such collection cylinder, the cylinder is unscrewed, sealed and beetles are transferred into rearing boxes.

Each rearing box contains a food source of honey and pollen, and also a pair of glass microscope slides, glued face-to-face, to provide a crevice into which beetles can lay their eggs (Figure 9). Within a few days each slide 'sandwich', containing numerous eggs, is removed and placed into a fresh culturing container to allow larvae to hatch and grow (Figures 10 and 11).

As larvae reach the final stages of development, their behaviour changes and they begin to wander en masse. This signifies that they are ready to pupate, so they are transferred into a container filled to three-quarters depth (15 cm) with approximately 1.5 litres of damp sand. Wandering larvae rapidly dig down into the sand where they pupate (Figure 12), later (after approximately 33 days at 20 °C) to re-emerge as the next generation of adult beetles.

RESEARCH PROJECTS THAT UTILISE THE SHB CULTURES

The cultures of SHB held by Fera are currently being used in a series of population development studies, designed to help us understand the overwintering potential of this pest in the UK. We are also working with larvae to assess the efficacy of a variety of pest control methods against the immature life stages of SHB. Cultures have been used extensively by Fera and NBU staff to develop and test chemical attractants, for use as lures to baited traps for the purposes of monitoring and controlling beetle numbers.

Full details of these and other Research and Development projects relating to honey bee health can be viewed on our BeeBase website at www.nationalbeeunit.com

HOW BEEKEEPERS CAN HELP KEEP SHB OUT OF THE UK

Be vigilant – keep an eye out for SHB in your colonies. Look at available information to help you understand the beetle's biology and signs of its presence (see details in our leaflet, *The Small Hive Beetle*, freely available through BeeBase (www.nationalbeeunit.com) or from the NBU).

Send suspect samples to the NBU immediately – details of how to do this are also available from BeeBase.

If you are an importer of bees, it is really important that you maintain accurate records which allow you to keep track of the whereabouts of your imports. Full guidance regarding the importation of bees can also be found on BeeBase.



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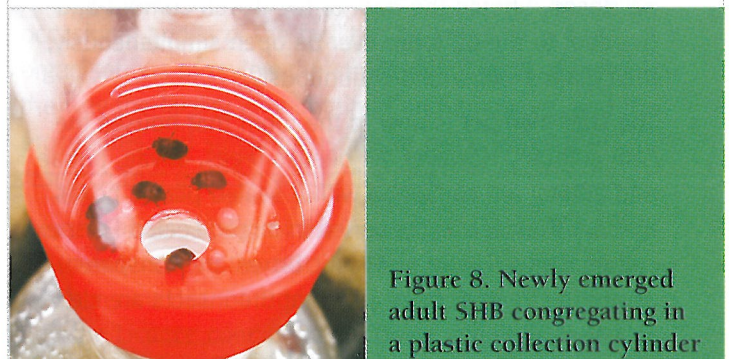


Figure 8. Newly emerged adult SHB congregating in a plastic collection cylinder



Figure 9. An oviposition site, made of two glass microscope slides, containing SHB eggs

Import regulations are our main defence against the introduction of SHB (and other very serious bee pests and diseases) from overseas to the UK – it is absolutely essential that all beekeepers abide by them.

Become a registered beekeeper – it is extremely important that all beekeepers register on BeeBase. If we don't know where 'at risk' apiaries are located, then our chances of effectively monitoring for the arrival of SHB, or achieving control in event of an incursion, are seriously jeopardised. This is the responsibility of the beekeeper.

To register as a beekeeper, please visit www.nationalbeeunit.com

FURTHER INFORMATION

Dr Gay Marris is Science Writer for the NBU. Dr Andrew Cuthbertson, Senior Entomologist, along with James Mathers and Lisa Blackburn, coordinates the Applied Entomology Quarantine-based research within the Crop and Food Security Programme at Fera.

Please send any enquiries about honey bees to nbu@fera.gsi.gov.uk. For enquiries regarding Bee Health Policy and Regulatory issues, contact Bee Health Policy at beehealthinfo@fera.gsi.gov.uk ◆



(clockwise from top left)

Figure 10. SHB larvae hatching from glass slides

Figure 11. SHB larvae on sand

Figure 12. SHB pupating in the sand

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