# FINAL REPORT FOR PROJECT PH0506 – ANNEX 1

## Objective 1.3 Investigate feasibility of using available chemical and biocontrol products (e.g. nematode- or fungal-based) for SHB management in the UK.

## *Milestone 3: Review of available chemical and biocontrol methods (entomopathogenic fungi & nematodes) for use against immature stages of Small Hive Beetle (Aethina tumida) in the UK*

**Introduction**

The Small Hive Beetle (SHB), *Aethina tumida*, is a parasite and scavenger of honey bees (*Apis mellifera* L.). SHB is native to sub-Saharan Africa. In its native range, SHB is endemic and causes relatively little damage (Brown and Morton 2003). However, SHB has become an invasive species and its global range extends to North America, Egypt and Australia, and has caused considerable damage to European *Apis mellifera* (Neumann and Elzen 2004). The effect on apiculture economics and honey bee colonies in the USA and Australia can be severe (Ellis *et al*. 2002, Hood 2000, Levot and Haque 2006).

With increasing international trade of bees and bee products, the risk of SHB becoming established in the UK is growing (Cuthbertson and Brown, 2009; Cuthbertson *et al*. 2010; Marris *et al*. 2010). This was highlighted when SHB was intercepted in Portugal in 2004 and a further suspected case in 2006. Here we review control methods for SHB in use in countries with SHB, with a view for application in the event that SHB establishes in the UK. We focus on commercially available control methods and explore studies on potential chemical and biological controls.

**Chemical and Biological control**

Buchholz, *et al* (2006) demonstrated that chemical treatment with Perizin is an effective chemical control treatment for SHB, whilst *Bacillus thuringiensis* bacteria were ineffective. *Bacillus thuringiensis* (*Bt*) are used for pest control in a range of commercial products. The *Bt* toxins are species selective.

The efficacy of the disruption of the SHB breeding cycle was tested using three products containing different *Bt* strains; **B401®** (delta-endotoxins of *B. thuringiensis* var. *aizawai*, stereotype 7); **Jackpot®** (*B. thuringiensis* var. *kurstaki*) and **Novodor®**(*B. thuringiensis* var. San Diego *tenebrionis).* **Perizin®** (3.2% Coumaphos) was also tested. Adult beetles were allowed to feed, mate and oviposite on treated combs. Treatment with Perizin significantly reduced the number of wandering larva compared to B401® and the control group. No significant differences in numbers of wandering larvae were found between treatments of Novodor®, Jackpot® and controls.

Perizin treatment effectively reduced SHB reproduction. However none of the *Bt* strains tested were efficient as a SHB control. This supports the strain-dependant insect selectivity of *Bt,* opening further investigation into the testing of more *Bt* strains against SHB.

Coumaphos is also the active ingredient in **CheckMite+®**. This product is commercially available and widely used for control of SHB in the USA. Elzen *et al*. (1999) demonstrated that SHB can be successfully controlled using Checkmite+® strips (10% coumaphos) in trapping devices. Neumann and Hoffmann (2008) demonstrated that although mortality at the colony level was limited, CheckMite+® strips can be effectively used for estimating SHB infestation levels when applied in bottom board traps.

Studies by the Australian government have shown the potential of a trapping device treated with dilutions of the insecticide, fibronil (commercially available in **Regent 200SC®**) as an in-hive control measure against SHB. Laboratory experiments identified fibronil from eight insecticides as the most potent to SHB. Tests demonstrated a 98.4% mortality of SHB compared to controls (Levot and Haque 2006) and further development of the trap has shown favourable results in honey residue trails (Levot 2007). However, registration and commercialisation of this product is reliant on a commercial interest by a company or other body and complications with patient rights. Interestingly, of the other insecticides tested, temephos, imidacloprid and methomyl showed effectiveness similar to or better than coumaphos.

**Gard star®** is a chemical treatment registered in many states in North America for use against SHB. The product is a liquid treatment containing 40% perimithrin and is used as a soil drench to kill SHB pupae (Hood 2000). The beekeeper dilutes the product with water and drenches the soil underneath bee colonies.

**Organic acids**

Schafer *et al*. (2009) demonstrated that formic acid significantly increased SHB mortality and therefore treatments with organic acids could become part of an integrated pest control programme for SHB.

Laboratory experiments tested the effects of five organic acids (formic, acetic, lactic, oxalic and citric acid) on the SHB associated yeast, *K. ohmeri*. Treatments were diluted in water and, with the exception of citric acid, applied at concentrations similar to standard apicultural methods to control existing pests. Treatments of formic acid and acetic acid completely inhibited yeast growth and so these two acids were then tested in, in-hive-experiments against controls treated with distilled water.

Treatments were applied to a sponge placed inside nucleus colonies, again, at concentrations used in normal apicultural practices. Field treatments with formic acid (60%) significantly increased SHB larvae mortality whilst treatments with acetic acid (70%) significantly increased SHB adult mortality compared to controls.

**Lime and Diatomaceous earth**

Buchholz *et al*. (2009) tested the potential of dry slaked lime, powdered limestone and diatomaceous earth (**Fossil Shield®**) to control SHB. Tests were conducted to investigate efficacy as in-hive-treatments and to investigate pupation success of SHB larvae in the ground.

Laboratory tests observed the effect of treatments on SHB larvae pupation in soil cups. Treatments were applied at different dosages across all three test substances. Field tests were also conducted by applying test substances to traps placed in bee colonies.

While slaked lime hindered pupation, treatment with high doses of Fossil Shield® significantly increased SHB mortality. No significant differences of SHB mortality were observed in laboratory treatments of powdered limestone. This indicates that diatomaceous earth products have potential as alternative in- hive chemical control of SHB.

**Fungal Control**

Muerrle *et al.* (2006) and Ellis *et al.* (2004) showed potential for fungal control of SHB. Ellis et al investigated the mortality effects and identified fungal pathogens that appeared to increase SHB larval mortality. SHB exposure was achieved by; 1) ingestion of honey bee brood inoculated with an emulsion of the dead, colonised larvae. 2) post-feeding contact to dead SHB larvae that were colonised by fungi.

SHB mortality was significantly higher when larvae had been exposed to fungi post feeding. No treatment effects were observed in the ingestion experiment. Two species of Aspergillus were identified on the cadavers, *A. flavus* and *A. niger.* However, further investigation is needed to establish if these two species are causative agents of SHM mortality. Muerrle *et al*. (2006) evaluated the susceptibility of SHB to *B. bassiana*, *H illustris, M. anisopliae* and *M. anisopliae* variety *anisopliae.* The fungal genera *Beauveria, Hirsutella and Metarhizium* are general entomopathogenic fungi. Some strains are available commercially and are used successfully to control a range of insect and mite pests.

SHB were collected from and reared in South Africa. Viable spore suspensions were fed in sugar patties to SHB in petri dishes. Mortality of SHB was significantly higher with treatments of *M anisopliae* variety *anisopliae* and *B bassiana* compared to controls. No significant differences in mortality were shown between controls and treatments of *M. anisopliae* and *H. illustris*. *Beauveria bassiana* significantly increased SHB mortality above all other treatments. However, preliminary studies on SHB larvae produced a lower mortality. This confirms previous studies that suggest infectivity is linked to specific insect developmental stages.

**Nematodes as control agents**

Cabanillas and Elzen (2006) and Ellis et all (2010), demonstrated that entomopathogenic nematodes could be an effective component of an integrated pest management scheme for SHB.

Cabanillas and Elzen (2006) demonstrated treatments of *S. riobrace* had a high efficacy against prepupal SHB larvae. Three commercially available entomopathogenic nematodes were tested; *Steinernema carpocapse* and Heterorhabditis *megids* from ARBICO Environments and *S. riobrave* from USDA –ARS, KSARC, Weslaco, TX. Nematodes were introduced in soil substrate at four concentrations to wandering SHB larvae. Treatments with *S. riobrace* gave a lower LC 50 than the other nematodes and no SHB mortality occurred in control groups. Ellis et al (2010) advanced the work of Cabanillas and Elzen (2006) investigating the efficacy of entomopathogenic nematodes species and concentration.

Seven nematode species and ten strains, in total, were tested. Four separate laboratory tests, a field test and a general nematode persistence test were conducted. Each laboratory test used different substrates; two soil types; sand and filter paper. Each laboratory test used a different number of nematode strains at different concentrations. One laboratory test used seven-day-old pupae while the rest used wandering larvae. Another laboratory test investigated possible effects of two temperatures, 20C and 25C. Another test investigated longer term SHB mortality by investigating the effects of six nematode strains for 6 days, then selecting three nematodes to continue testing for 39 days. Treatments were applied using nematode infected juveniles of wax of larvae. Juveniles were infected at different levels.

In the field trial, two nematode strains were tested in forested and clear field areas that were periodically wetted or left for natural rain fall. In the general persistence test, two nematode strains were tested with two different application methods to determine whether single soil inculcations would provide continued control. Nematode efficacy varied significantly by tested nematode and the infective juvenile level at which they were applied. *S riobrave* and *H Zealandica* significantly increased SHB mortality over the controls in the first laboratory test. In the forth, *H bacteriophora* and *H indica* significantly increased SHB mortality after nine days and almost 100% SHB mortality was observed across all three nematode treatments (*H bacteriophor, H indica* and *S riobrave*) after 39 days. No other laboratory test showed significant SHB mortality against controls.

In both the general persistence test and field trails, *H indica* and *S riobrave* significantly increased SHB mortality. Location had a significant effect on SHB mortality with more SHB emergence from the forested site than the clear field site. No significant differences in mortality were observed between moisture, and methods of soil inoculation.

**Products available in the UK**

The risk of SHB establishing in the UK remains high. Studies have shown the potential for alternative treatments such as biological fungal control, entomopathogenic nematodes, organic acids and diatomaceous earth. However further work and eco toxilogical trails are needed to take some of these treatments to registration and make them commercially available. Of the successful commercially available products discussed, Checkmite+® and Perizin ® are both currently obtainable for use in the UK, under a special import licence, via the Veterinary Medicines Directorate, under the Cascade agreement. Organic acids are used by UK beekeepers; acetic acid is used as a fumigant for stored boxes and combs and formic acid can be used to treat for varroa, under prescription. Both organic acids have been shown to have an effect on SHB survival, indicating a possible method of control available in the UK. In the current study Checkmite+® has been chosen for trial.

In regards to entomopathgens the range of commercially available products to use is relatively small. A few of the agents available, for example the nematode *S. feltiae* has been shown to have little impact from work in the USA. The following agents that are available 'off the shelf' in the UK and that have the potential to offer some level of control will be tested in the current study are as follows:

- Entomopathogenic nematode: Nemasys L (*Steinernema kraussei*)
- Entomopathogenic fungus: Naturlais-L (*Beauveria bassiana* ATCC 74040)

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