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Small Hive Beetle Biology

— Producing control options —

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Small Hive Beetle Biology

Providing control options

by Nicholas Annand

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Foreword

The Small Hive Beetle (SHB) was first identified in Australia in 2002. Since then it has become a major pest of honeybee hives. When conditions are suitable, beetles lay their eggs within bee hives and honey sheds, often in the combs, either in the hive or in stored honey combs pre- or post-extraction. The hatching larvae then feed on the honey, pollen, bee eggs, bee larvae and brood. The resulting contamination of the honey renders it useless for extraction, thereby leaving the beekeeper bereft of their main source of income from the hives. Beekeepers also face the costly exercise of cleaning up contaminated supers and hives and restoring colonies to full strength following heavy infestations.

Many control strategies are already in use in Australia to minimise the impact of SHB and include modifications to hive designs, improved beekeeping techniques and hygiene procedures. However SHB continues to cause large-scale economic losses within the industry. It is now clear that a better understanding of the biology of the SHB is necessary if beekeepers are to effectively manage this pest.

This project highlights the biological and behavioural characteristics of SHB that can be directly related to hive health and management. The knowledge can be used to enhance the effectiveness of current control strategies and to provide the basis for new and improved control strategies for the commercial and amateur beekeeping industry.

The findings of this report are aimed at supporting current and future management strategies for SHB in the beekeeping industry.

This project was funded from industry revenue which is matched by funds provided by the Australian Government.

This report is an addition to RIRDC's diverse range of over 2000 research publications and it forms part of our Honeybee R&D program, which aims to improve the productivity and profitability of the Australian beekeeping industry through the organisation, funding and management of a research, development and extension program that is both stakeholder and market focused.

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Craig Burns
Managing Director
Rural Industries Research and Development Corporation

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Abbreviations

ANOVA – Analysis of Variance

GLMM – Generalized Linear Mixed Model

I&I NSW – Industry and Investment New South Wales

LMM – linear mixed model

NSW – New South Wales

Qld – Queensland

RH – relative humidity

RIRDC – Rural Industries Research and Development Corporation

SHB – Small hive beetle (*Aethina tumida*. Murray)

UWS – University of Western Sydney

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Executive Summary

What the report is about

The report examines some key aspects of SHB biology and behaviour that may lead to new and improved control strategies for this recognised pest confronting the apiary industry.

Who is the report targeted at?

The report targets beekeepers, particularly those with hives heavily impacted by SHB, and extension staff in advisory roles. It is intended to be a basis for further research and development into improving the sustainable management strategies for SHB for the Australian apiary industry.

Where are the relevant industries located in Australia?

Currently SHB is found in New South Wales, Queensland, Victoria, the Australian Capital Territory and a small population in the Kimberley region in Western Australia. The eastern states comprise the majority of the apiary industry in Australia, with NSW home to about 40% of Australian hives. SHB has had the greatest impact in those areas where the climatic conditions better suit its survival, in particular the warm and humid coastal strip between the NSW south coast and southeast Queensland. However the affects of the SHB continue to spread and expand west, increasing its nuisance value particularly when seasonal conditions favour their breeding.

Background

Since the introduction and identification of SHB in Australia in 2002 this pest has spread rapidly throughout the Eastern mainland states. SHB has since caused major economic losses to apiarists through reduced productivity.

Prior to 1998 SHB went largely unrecognised in its native homeland of sub-Saharan Africa. It was considered a minor pest to the African bees and attributed to minimal economic losses. However its identification in the USA, followed four years later in Australia, raised its profile rapidly to a pest of major economic significance for beekeeping in both countries. The past twelve years have seen an extensive increase in research on SHB. This report aims to build upon this research.

Aims/objectives

This research aims to improve our understanding of several biological and behavioural features of SHB, and how these impact with localised environmental factors such as temperature, humidity and seasonal variation. In particular this research focuses on:

1. Determining the temperature and humidity thresholds for SHB egg laying and emergence

2. Comparing the relative attractiveness and susceptibility of hives of differing strength to SHB infestation and damage

3. Identifying any patterns in movement of SHB in and out of hives over a year

4. Relating numbers of SHB found within hives to the number found in the immediate environment, and

5. Investigating the effectiveness of a range of treatments on ‘slimed’ (SHB larvae-damaged) combs in terms of cleaning and the subsequent willingness and ability of bees to repair any damage.

Methods used

With a variety of areas investigated in this project, different methods were used for each section.

Trial 1 involved laboratory reared SHB being placed into temperature and humidity controlled environments. This work was conducted at the Industry & Investment NSW Research Station at Bathurst.

For **Trial 2** hives were manipulated to create varying hive conditions (strong, weak and queenless) in a relatively SHB free location (Bathurst). They were then relocated to an area at the University Western Sydney (UWS), Richmond known for high SHB numbers and exposed and monitored in that environment over 10 weeks starting 1 February 2008.

In **Trial 3** four hives were located at UWS Richmond with digital recording cameras' monitoring SHB activity at the hive entrances for 24 hours, around the beginning of each month, for over a year. Movements of SHB in and out of the hives were monitored and the populations within each hive were counted after each recording.

Trial 4 was performed using six two decker bee hives at the UWS Richmond. Metal enclosures were made and used to cover the ground immediately after the hives were moved forward by a metre. The SHB under the hives and those collected in lures within the enclosures were compared with what was collected and counted from within the hives. This was done monthly for over a year.

For **Trial 5** a variety of simple washing techniques were used to treat slimed combs. These combs were then returned into hives located at Bathurst. The frames were monitored on several occasions to assess how successful the washing techniques and the bees were in cleaning the treated frames for reuse.

Results/key findings

Environmental conditions of temperature and relative humidity were investigated to identify threshold values for SHB egg laying and emergence. It was found that temperatures of less than or equal to 15ºC and greater than or equal to 45ºC prevented SHB from laying eggs. In addition eggs exposed to these temperatures did not hatch. Relative humidity of less than or equal to 34% also prevented egg survival.

Healthy, strong hives attracted more SHB than hives in a weakened state. It was also shown that queenless hives were no more attractive or susceptible to SHB when bee populations were high. However when hive populations decreased to very low numbers, as a direct result of being queenless, these hives became far more susceptible to SHB damage.

Over the 24 hour periods the greatest number of SHB entering hives occurred in the two hours prior to nightfall. Most SHB movement occurred in the autumn months of April and May however a major spike was also observed in October. The populations of SHB in the hives peaked in late autumn then declined right through winter to bottom out in late spring producing a very cyclical pattern.

Almost half the SHB observed were outside the hive during the hottest month of the year however when seasonal conditions cooled the SHB retreated back into the hive.

Washing 'slimed' combs made no difference to the bee's ability to clean and resurrect slimed material. The trial did show that slimed combs can be resurrected by the bees when returned to the hives cautiously.

Implications for relevant stakeholders

These findings support practical changes in beekeeping based on science. Apiarists can use temperature and humidity thresholds as a guide to better management of the extraction shed and stored supers. Knowing when SHB are most likely to move during the day, and when their numbers in the hive have the potential to be at their greatest allows apiarists to adjust their management accordingly. This may mean using chemical control methods at the most advantageous times or more closely monitoring hives when they are most susceptible.

These findings enhance the potential effectiveness of control methods already used within the industry through an increased understanding of seasonal SHB population dynamics, movement activity, localised movement habits and the susceptibility of hives to SHB damage.

It is intended that by applying the information from this report beekeepers will be able to reduce the time and money spent on managing SHB, increase honey yields and therefore marketable product. The status of the industry as a whole will be far healthier with beekeepers remaining in the profession rather than turning elsewhere for economic dependence. Pollination-dependent industries will also be better supported by apiarists with strong healthy hives and this has far-reaching spin-offs into agricultural export markets and the community as consumers of food.

Recommendations

The information from this report can be used to implement SHB control strategies more effectively and develop new and improved targeted strategies to limit SHB larval damage. It also adds to the body of knowledge on SHB that can be used by researchers in the future.

This report provides information that promotes methods of long-term and sustainable SHB pest management.

Introduction

Originally from sub-Saharan Africa, the small hive beetle *Aethina tumida.* Murray (SHB) attracted minimal interest on its home continent. The beetle had evolved alongside the endemic bee populations and the beekeepers who managed them. Damage was minimal and economic losses were small enough to avoid real concern. However more recently SHB has been identified in many larger honey-producing countries including the USA, Canada and Australia. Beekeeping practices in these countries are different, as are the sub-species of bees used in the majority of apiaries. As a result SHB has become a pest of significance in these countries, with large economic losses being attributed to its presence.

SHB is now considered a major pest of the apiary industry in Australia, particularly in the warm and humid environments along coastal NSW and Queensland. Anecdotal evidence from a survey of beekeepers along the NSW north coast found average losses of around 10% of hives due to SHB. Interestingly hives can withstand high numbers of SHB adults, with minimal damage being noted as a result of their infestation.

It is the reproductive phase of the SHB lifecycle which is particularly destructive. When adult SHB are able to lay their eggs it is the emerging larvae that chew or tunnel through the comb, consuming brood, pollen and honey along the way (Lundie, 1940; Schmolke, 1974). Whilst feeding the larvae also carry a yeast, *Kodamaea ohmeri* (Torto *et al.*, 2007) which contaminates the honey and causes it to ferment. This 'sliming' is a clear symptom of SHB damage. Once heavily infested with feeding SHB larvae the bee colony will typically abscond from the hive (Suazo *et al.*, 2003; Neumann and Elzen, 2004), leaving the remaining hive material completely vulnerable to attack. An estimated 6000 SHB larvae can be produced on a single frame of brood (Brown *et al.*, 2002) and the larvae will spread across the unguarded combs.

European honeybees have a number of defence strategies that can help the hive to minimise SHB damage. They have been seen to imprison SHB and keep guard over them (Ellis, 2002; Ellis, 2003b; Ellis *et al.*, 2003b; Ellis, 2003a), aggressively try to bite and sting the SHB (pers. observation), remove SHB eggs (Ellis *et al.*, 2004; de Guzman *et al.*, 2008; Ellis and Delaplane, 2008), remove SHB larvae (de Guzman *et al.*, 2008) and guard hive entrances against adult SHB.

Unfortunately these defence strategies are not always enough. Any situation or factor which reduces the ability of the bees to guard their comb leaves the colony or hive material vulnerable to attack (Lundie, 1940). This includes stored unguarded frames in the honey shed, before and after honey extraction or just stored material. With no bees in residence to defend the frames the SHB larvae have an opportunity to spoil the honey in the final stages before extraction and sale.

Lamb (2010) estimated that losses to Queensland beekeepers were around $2 million per annum and that Australia-wide this figure would be closer to $4.5 million. These losses include costs associated with 'slimed' honey, damaged and destroyed hives, weakened and lost colonies, SHB control, increased management and time and lost potential honey yields. Therefore the need to develop a better understanding of SHB and their interaction with European honey bee colonies is crucial.

Over the past ten years the amount of research into SHB has increased dramatically, a true indication of the level of importance now placed on this pest within the apiary industries around the world. However, there is still a long way to go before SHB is understood to a point where we can truly say we know how to manage it effectively.

Objectives

This research aims to improve our understanding of several biological and behavioural features of SHB, and how these impact with localised environmental factors such as temperature, humidity and seasonal variation. In particular this research focuses on:

1. Determining the temperature and humidity thresholds for SHB egg laying and emergence
2. Comparing the relative attractiveness and susceptibility of hives of differing strength to SHB infestation and damage
3. Identifying any patterns in movement of SHB in and out of hives over a year
4. Relating numbers of SHB found within hives to the number found in the immediate environment, and
5. Investigating the effectiveness of a range of treatments on 'slimed' combs in terms of cleaning and the subsequent willingness and ability of bees to repair any damage.

Much information in the apiary industry is based on anecdotal evidence, as gained from beekeepers working their hives for a living. However this evidence is often conflicting, it is particular to certain conditions and sites, and evaluation of the success of management techniques in controlling SHB is hard to survey and quantify accurately. This research aims to provide data that can then be used to develop integrated pest management plans for SHB in the Australian situation.

Five research trials were conducted and the objectives for each are listed below. Each trial has been reported as a separate unit.

Trial 1

To determine the temperature and humidity thresholds for egg laying and egg hatch.

To provide beekeepers with sound recommendations on techniques and/or appliances that could be used practically in honey sheds to achieve environmental conditions to inhibit SHB damage to stored honey supers (fulls and stickies).

Trial 2

To compare the relative attractiveness and susceptibility of hives to SHB when in different health states, ie. strong, weak and queenless.

Trial 3

Monitor and record SHB movements in and out of healthy hives over a 24 hour period once a month over a year. Correlate the observed SHB movement times with the environment temperature and humidity and compared the number of SHB within the hive.

Trial 4

Determine the proportion of the SHB found in close proximity outside the hive and ascertain if this fluctuated throughout the year.

Trial 5

To determine whether soaking slimed combs in water, pressure hosing, or washing in dilute bleach solution offered any benefit over no treatment, in terms of the subsequent willingness/ability of bees to re-use damaged comb.

Trial 1 – Conditions for Small Hive Beetle to Thrive

Methodology

A colony of SHB was established using adults obtained from the laboratory colony at Menangle and supplemented regularly with "wild" SHB collected from hives. The SHB were kept in 5 and 9 L clear plastic containers with lids containing many small ventilation holes. Damp paper towel was put on the floor of the container and scrunched up into a ball for a harbourage for the SHB. The adult SHB colonies were maintained on a diet of white sugar (sucrose) provided in a small plastic container. The SHB were kept in the dark inside a cabinet in an air conditioned work room with a temperature range between 15 to 26°C. To increase numbers, priming the SHB (as described below) provided an adequate number of eggs. All adult SHB were removed from the container and the eggs allowed to hatch. The larvae were fed with beekeeper collected honey bee pollen (which will be referred to as pollen) and a piece of comb containing some pollen and honey. The larvae were fed every few days ensuring surplus food was always available. The quantity of food varied depending on larval numbers and stage of development. Once larvae were mature and in the wondering phase ready to pupate they were separated from the food and frass and placed on top of moist soil or sand in cylindrical containers (10 cm diameter and 15 cm depth) into which they burrowed. These were then placed into a larger (9 L) lidded container with moist paper towel to maintain humidity and held at ~ 25ºC, A tablespoon of white sugar (crystals) was provided to feed the young SHB adults emerging in the container. Each batch of emerged SHB were kept separate in a container and the date of the last emerging SHB recorded. This ensured appropriately aged SHB were used for the trial.

Priming SHB

Around 90 randomly collected SHB between the age of 10 days to 2 months post emergence were placed in a plastic container (750 ml) with some pollen (as a protein source), a piece of comb containing pollen and honey and a piece of moisten paper towel. With excess food for the SHB, the container was then placed in an incubator maintained at 32°C for 24 h. This primed the SHB into egg production, with eggs visible in the container after the 24 h. The primed SHB were then used immediately in the trials.

SHB ready for trial

Into a 110 ml clear plastic container, was placed a 20 ml lid with 0.7 g. of pollen. On top of the lid sat two glass microscope slides that had been cut so they fitted inside the plastic container with one edge sitting on the lid in the base (Figure 1). The two slides were squeezed together with some Bostik Blu-Tak™, leaving a gap of approximately 0.5 mm between the slides for SHB oviposition. Ten randomly selected primed SHB were collected using a pooter and placed in each of eight containers with the slides, lid and pollen. A piece of nylon flyscreen was then placed over the container and a rubber band placed over the flyscreen holding it in place to contain the SHB.

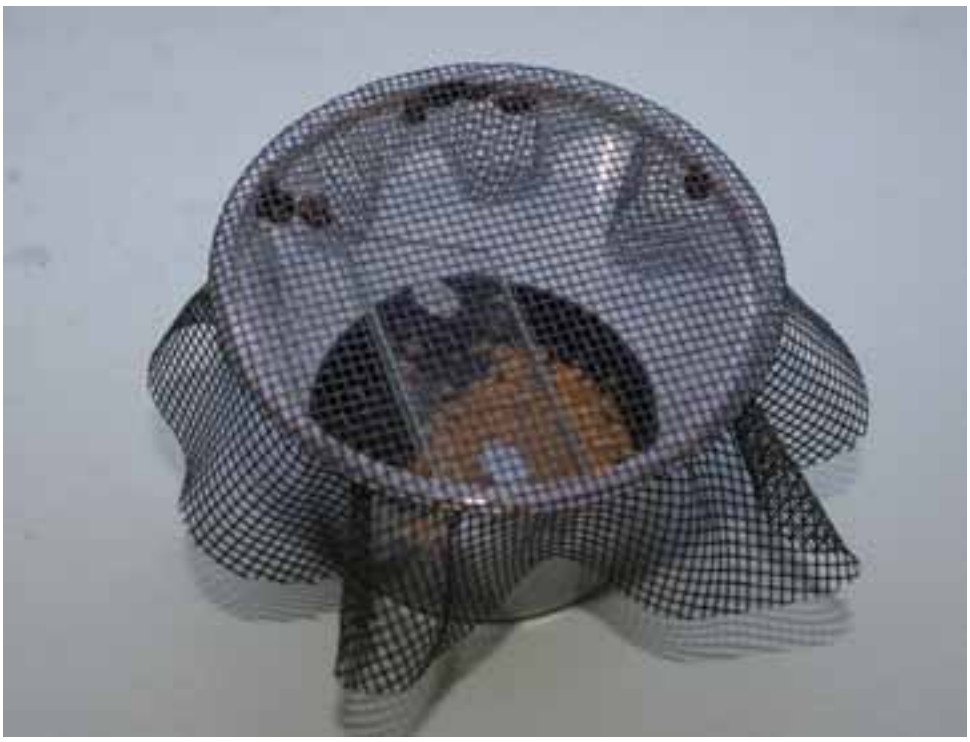

Figure 1 Container holding 2 glass slides, a lid with some pollen and 10 primed SHB

Controlled environment

An incubator with no cooling capabilities was placed in a cool-room to obtain the required temperatures (15°C to 45°C). Humidity was controlled by using various saturated solutions (Winston and Bates, 1960; Rockland, 1960) in 1.9 L plastic airtight food containers as desiccators. Four desiccators were fitted with digital hygro-thermometers. The sensors for these units were inserted through the desiccators' lids and the holes sealed with silicone sealant. The display units for the hygro-thermometers were located outside the incubator (Figure 2).

Figure 2 Desiccators containing saturated solutions within an incubator within a coolroom. Hygro-thermometer sensors were wired into the desiccators.

Humidity effect

Humidity was controlled using various saturated solutions or honey. For each solution 1.5 L of deionised water was heated to around 70 to 80ºC in a beaker and pure compound was added to, and mixed into the water. Compound was added and stirred over heat, but not boiling, until the water could not dissolve any more and residual compound remained. Three litres of deionised water was used to make up the glucose. The saturated solution was then stored in a sealed container to prevent any contamination until required for use in the desiccators. Compounds used were d-glucose anhydrous ($C_6H_{12}O_2$), magnesium chloride ($MgCl_2.6H_2O$) and potassium carbonate anhydrous (K_2CO_3). The four relative humidities (RH) used in the desiccators at 30°C, were 34% $\pm$ 2% ($MgCl_2$), 46% $\pm$ 2% (K_2CO_3) approx. 58% (honey) and 84% $\pm$ 2% (glucose).

Each desiccator was approximately a quarter filled with the appropriate saturated solution. Plastic ice cube trays cut to size with large holes cut into each cube were used as a platform to keep the containers holding the beetles out of the saturated solution while ensuring maximum air/solution contact. This hastened the return to equilibrium of the humidity within the chamber after opening.

Two containers, with ten primed SHB in each, were randomly placed into each of the four desiccators in the incubator. The two containers within the same desiccators were considered as one replicate (pseudo replicates) and numbers for both containers were combined. Thirteen replicates in time were done for each of the four different humidities.

The hygro-thermometers where reset about 30 to 60 minutes after putting the SHB in, allowing time for the temperature and humidity to return to equilibrium. The SHB were removed after 24 h, at which time the SHB were separated into sexes by gently squeezing between the thumb and forefinger. For females the ovipositor extended quite a way out and for males an additional eighth tergite, which females do not have, protruded from the posterior (Schmolke, 1974). No beetles were re-used. The total number of eggs laid were counted with differentiation of eggs laid between the slides (Figure 3) and eggs found else where in the container. All eggs were counted even if they appeared unviable. Counting was performed under 12 X magnification using a stereo microscope. After counting, the slides were returned to the containers (less beetles and flyscreen) and placed into the same desiccators within 30 mins. The proportion of hatched eggs between the slides was determined two days later.

The humidity levels for the saturated solutions were effectively constant. The honey had some variation in humidity levels and not being a saturated solution a slow decline in humidity due to opening and closing the desiccator was experienced. In trying to maintain constant humidity, water was added to rectify the decline and on occasions this was over done causing variation in the humidity. The humidity varied from as high as 74% down to 54% but most (9 of the 13 replicates) occurring within 57% and 60% RH.

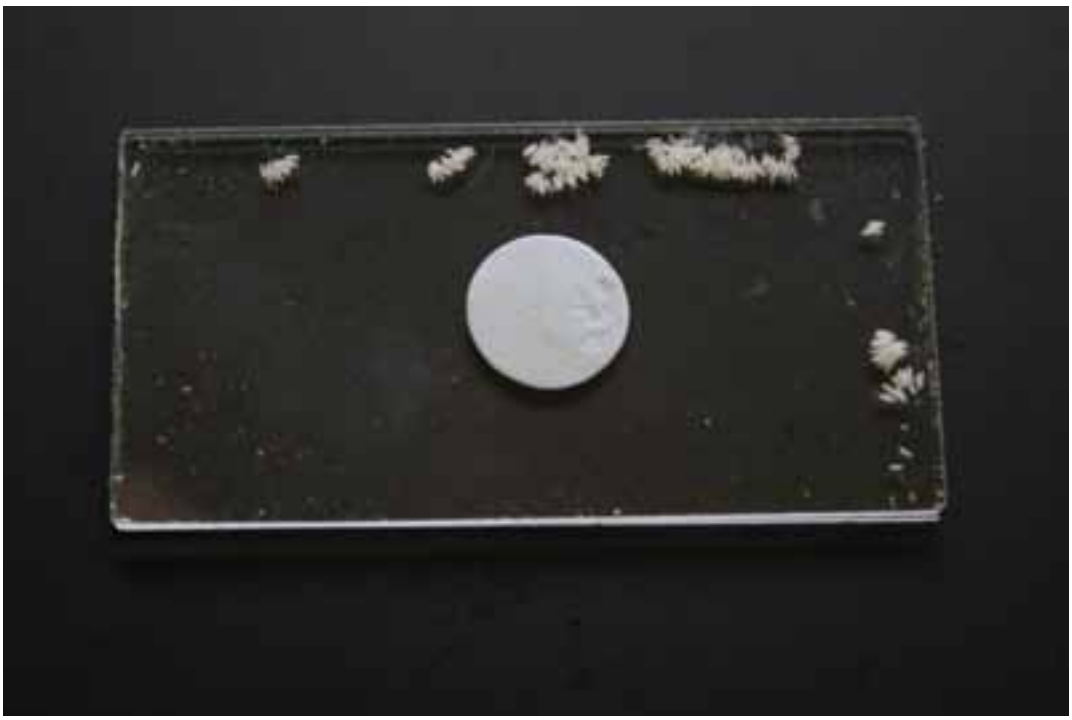

Figure 3 Two slides held apart with Blu-Tak™ with SHB eggs oviposited between the slides.

Temperature effect

The trial design was similar to that described above but the humidity was kept constant (80 to 85%) by using four desiccators containing a glucose saturated solution across a varying range of temperatures. Incubator temperatures were set from 15°C to 45°C with 5°C increments. Except for the trials conducted at 30ºC all replicate trials for each temperature were done consecutively. The trials at 30ºC were a sub-set of the humidity trial above and were replicated 13 times. For all other temperatures examined, four replicates were done at one time in the incubator. For 15°C and 45°C this was done on four different occasions (ie four replicates in time) with a total of 16 replicates. For 20°C, 25°C and 35°C there were five replicates in time (total 20 reps.) while for 40°C six replicates in time were used (total 24 reps.).

Due to inadequate eggs being oviposited by SHB at 15ºC and 45ºC meant eggs had to be obtained by alternate means to test egg survival. For all other temperatures tested oviposition provided adequate eggs to examine egg hatch. Eggs for 15ºC and 45ºC were attained by overnight (18 h) priming, and with 15 to 20 SHB used in each container. The SHB were only given 3 to 6 h to oviposit between the slides at 32ºC. The SHB adults were removed and the eggs between the slides counted and placed into desiccators at 80%+ RH at 15ºC or 45ºC as done for the rest of the trial. This technique produced more eggs in a shorter time decreasing the exposure of the eggs to 32ºC.

With development rates of the eggs influenced by temperature, times in the desiccators were reduced for temperatures greater than or equal to 35°C to 18 h to prevent eggs hatching prior to the initial egg count. Hatching eggs made counting extremely difficult and unreliable. Similarly the time allowed to ensure all viable eggs had time to hatch was extended for cooler temperatures out as long as five days at 15ºC with an additional two days at 26ºC and greater than 80% RH to make sure nothing hatched.

Data presentation and analysis

All (generalised) linear (mixed) model analyses reported below were performed using either ASReml (Gilmour *et al.*, 2006) or under R (R Development Core Team, 2009) using the package *asreml*

(Butler, 2009). Test of significance for fixed effects in the models are based on the methods developed by Kenward and Roger (1997).

Effects of temperature on egg laying

A linear mixed model was used to analyse these results where the different *temperatures* were included as fixed effects and temperature effect deviations across dates were included as random effects. The error variation (the variation across replicates within an incubator) was also allowed to differ across temperatures.

Note: Because only single replicates were done on any date for *temp* = 30ºC the error variance across dates at this temperature were confounded. So for this temperature the two sources were combined into a single source of variation, that being error.

Effects of temperature on eggs survival

The results for both *temp* = 15ºC and *temp* = 45ºC were 0 resulting in their exclusion from the analysis, as their inclusion would have only caused some distortion. The remaining results were analysed using a Generalized Linear Mixed Model (GLMM). The variable analysed was the number hatched which was assumed a binomial variate with parameters *N* (number of eggs on the slide) and *p* (proportion hatched). The parameter *p*, on the logit scale, was modelled as a linear combination of a *temp* effect (fixed) and a *date* and *temp* interaction effect (random), where the latter terms corresponds to particular incubators. When fitting the model, ASRemL an over-dispersion parameter was included.

Effects of humidity on egg laying

A linear mixed model was fitted to analyse the results. Humidity was included in the model as a quadratic (fixed effects) whilst date tested was included as a random effect. The error variance was fitted as inversely proportional to the number of female SHB (femshb). In these analyses the effect of the saturated solution compound used to create the required humidity's was ignored. Except for honey, saturated solution was totally confounded with the target humidity, whilst for honey where there was some variation in the relative humidities, there was to little data to detect a humidity trend.

Data was also analysed (ANOVA) comparing between the four treatments, ignoring humidity, after removing the *day/replicate* effect.

Effects of humidity on egg survival

The results were analysed using a generalised linear mixed model. The data, number of eggs hatching, was assumed binomially distributed (with possible over-dispersion) with parameters *N* equal the number of eggs on the slides and p the proportion of eggs hatched. The proportion p on the logistic scale was modelled as a quadratic model in humidity (fixed effects) plus a random date effect.

Results

Effects of temperature on egg laying

Temperature made a significant difference ($P < 0.001$) to the mean number of eggs laid per female SHB across the seven temperatures.

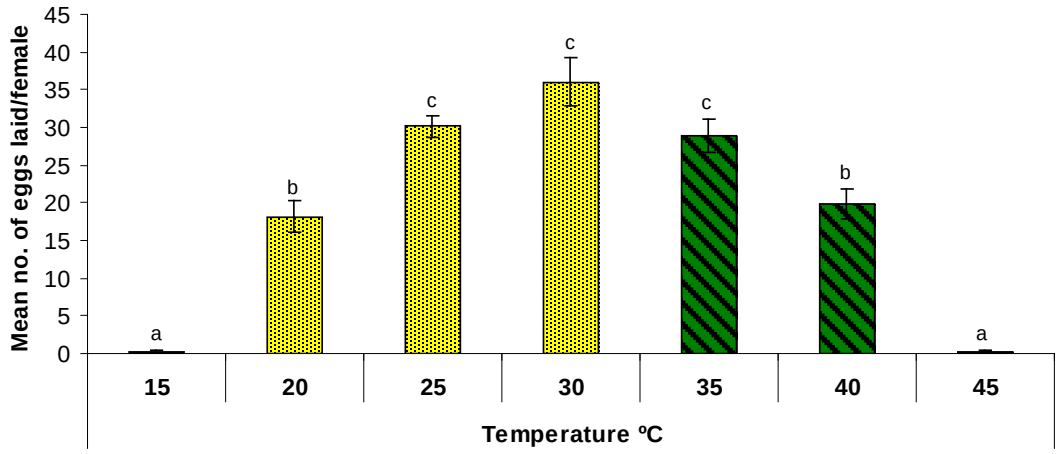

Figure 4 **The predicted mean number of eggs laid/SHB female over 24hrs (15ºC, 20ºC, 25ºC and 30ºC) or 18 h (35ºC, 40ºC and 45ºC) at different temperatures at 80% plus relative humidity. Bars represent the standard errors of the means and letters above the columns indicate the Least Significant Difference (LSD) ranking to 0.05 level.**

Effects of temperature on egg survival

There was a significant difference ($P < 0.001$) in the proportion of SHB eggs hatching across the temperature range. The two outlying temperatures, 15ºC and 45ºC, had zero eggs hatch, so were excluded from the statistical analysis but have been considered as significantly different to the others hence their inclusion in Figure 4. Approximately 30% of eggs hatched at 20ºC and 40ºC, with > 90% egg hatch for 25ºC, 30ºC and 35ºC.

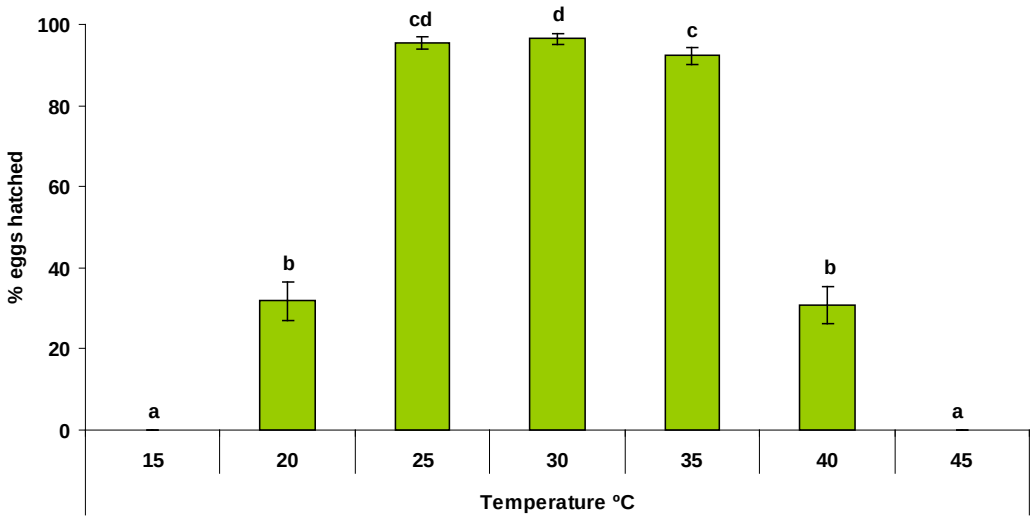

Figure 5. **The predicted mean percentage of eggs that hatch after being exposed to different temperatures at 80% plus relative humidity. Bars represent the standard errors of the means and letters above the columns indicate the Least Significant Difference (LSD) ranking to 0.05 level.**

Effects of humidity on egg laying

Increasing humidity caused a significant ($F_{1, 37.2}$= 65.3, P < 0.001) increase in the number of eggs laid per SHB female over 24 h. It also showed, with the addition of a quadratic effect for humidity, that the rate of increase in eggs laid per female significantly increased ($F_{1, 37.1}$ = 5.4, P = 0.026) as humidity levels increased. There was significant variation across dates.

From the linear mixed model the predicted eggs laid per female SHB was extrapolated for a range of humidities.

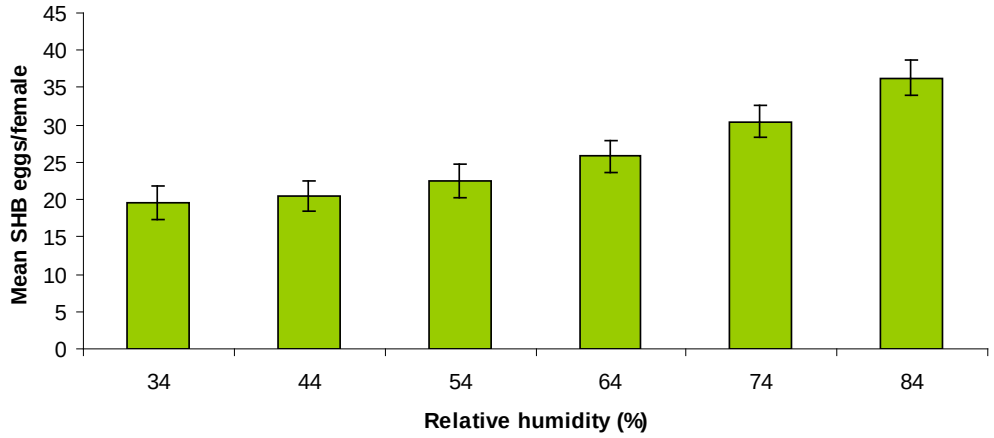

Figure 6. **The predicted mean number of eggs laid per female SHB for each replicate at different relative humidity's over 24 h. Bars represent the predicted standard errors of the means.**

An analysis across saturated solutions, ignoring target humidity and after removing day effects, showed significant differences ($F_{3, 36.1}$ = 22.97, P < 0.001) in the number of eggs laid per female SHB under each solution. Here saturated solution is a surrogate for target humidity.

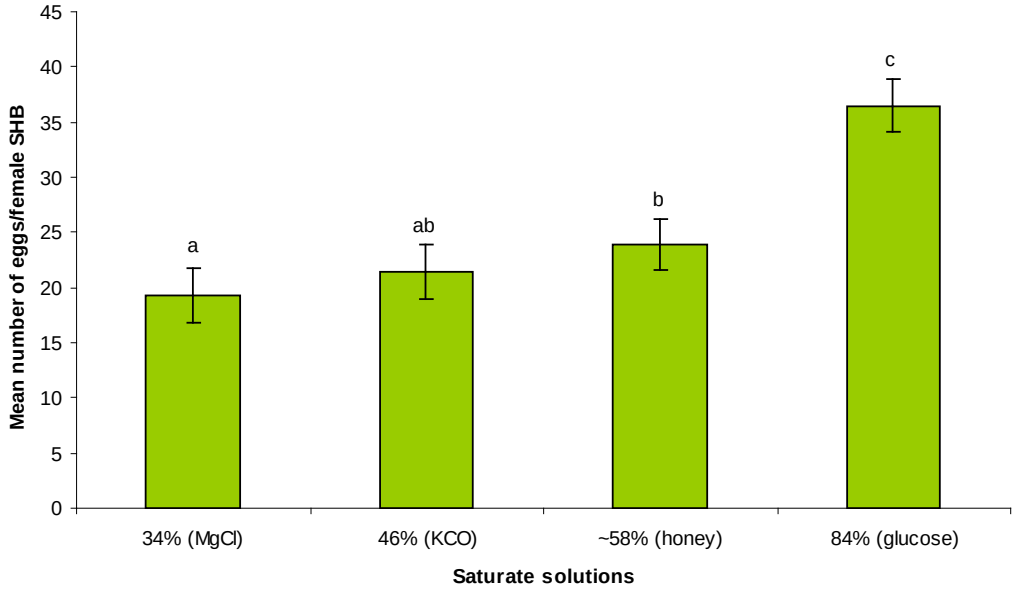

Figure 7. **The predicted mean number of eggs laid/SHB female over 24h in different humidity's at 30ºC. Bars represent the standard errors of the means and letters above the columns indicate the Least Significant Difference (LSD) ranking to 0.05 level.**

Effects of humidity on egg survival

After analysing the data using a generalised linear mixed model, the quadratic term, after removing the linear humidity effect, was significant ($P < 0.001$). There was also significant variation across dates.

The predicted values and associated standard error of percentage hatched for a selection of humidity levels are shown in Figure 8.

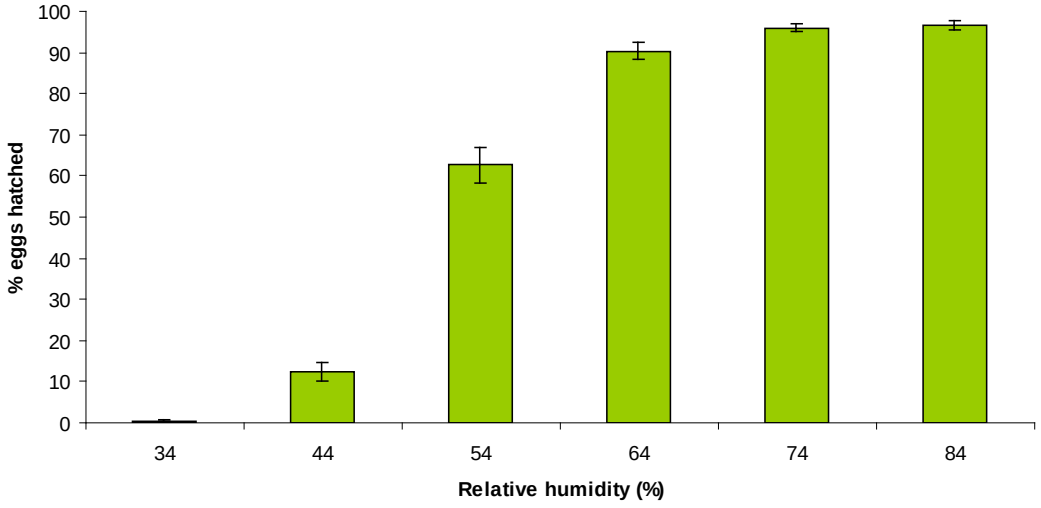

Figure 8. **The predicted mean percentage of eggs on slides that hatched after being exposed to different humidity's at 30ºC. Bars represent the predicted standard errors of the means.**

A Least Significant Difference (LSD) ranking at the 0.05 level showed significant differences in egg survival between all the target humidity levels tested. The honey did have some variation in the humidity levels but within these the results were still significantly different to those obtained at the other humidity levels.

Humidity had a major influence on the survival rates of SHB eggs. At 34% RH only 1 egg hatched out of 2186 eggs. It was observed when counting the eggs from the lowest humidity they had a deflated dried out appearance. These eggs desiccated and died. At the highest humidity (84%) almost 97% of eggs hatched. Very little deviation between replicates was observed in the proportion of eggs hatched at 34% and 84% humidity.

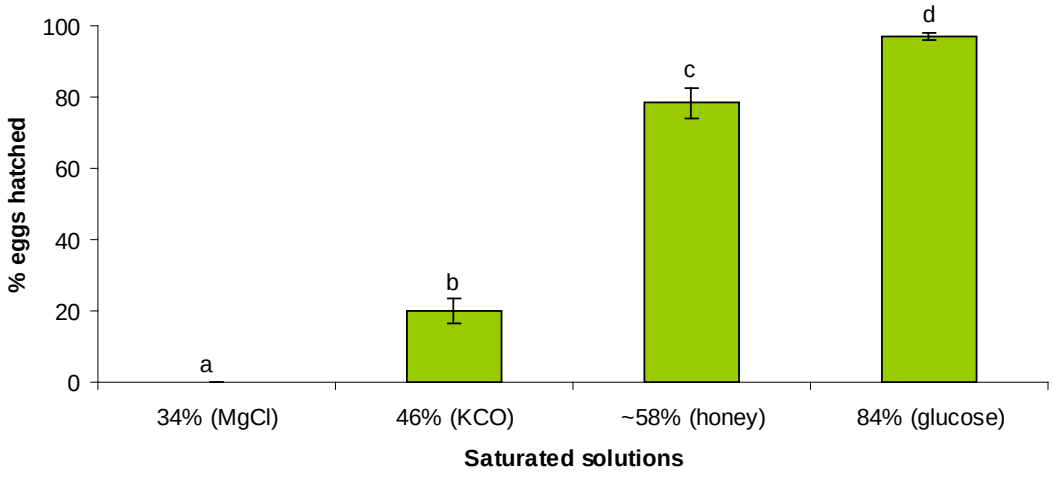

Figure 9. The predicted mean number of eggs that hatched on slides in different humidity's at 30ºC. Bars represent the standard errors of the means.

Discussion

When temperatures were less than or equal to 15ºC or greater than or equal to 45ºC SHB adults were prevented from laying eggs and SHB eggs exposed to these conditions did not hatch. At a relative humidity less than or equal to 34% SHB were capable of laying eggs however these eggs failed to hatch.

Egg production is usually maximal around the middle of the normal temperature range for most insects, perhaps representing a balance in utilisation of reserves into the metabolism of the insect and the production of eggs (Chapman, 1969). In a honey bee colony the brood area is kept around the 35ºC to allow development of the bee larvae (Graham, 2003). SHB are of Sub Saharan Africa origin (Hood, 2004) which is predominantly tropical. The optimal conditions for both SHB egg laying and survival were maximised at temperatures between 25 to 35ºC under high humidities, which closely correlates with what would be dominant in their natural environment.

For both the upper (45ºC) and lower (15ºC) temperature thresholds a very small number of eggs (less than or equal to 0.25 eggs/female) were laid on one occasion. It is suspected they were laid during set up or very shortly after being placed in the incubator. At high humidities the optimal temperatures for ovipositing fall between 25 to 35ºC, with the potential for SHB larval damage being very high within this range. At 20ºC and 40ºC there was a decline in the number eggs laid compared to 25 to 35ºC but there is still adequate egg laying to potentially cause major larval damage.

When comparing the results you need to recall that for temperatures greater than or equal to 35ºC, the time allowed for the SHB to lay eggs was shortened from 24 to 18h because eggs were hatching before 24 h. This was quicker than found by de Guzman and Frake (2007) who suggested egg incubation was around two days at 34 ºC. It was observed that all SHB adults when exposed to 45ºC, were dead when removed after 18 h. At 40ºC the majority of the SHB adults were still alive after 18 h but had become moribund, a condition not conducive to egg production. So reducing the hours from 24 to 18 h did not affect the number of eggs laid at 40 and 45ºC. At 35ºC a correction factor of $^4/_3$ to adjust for the time difference may give a truer indication of egg numbers (mean from 28.82eggs /female to 38.43eggs /female) but this is only speculation and will remain unknown unless tested.

As with eggs laid, the upper and lower temperature thresholds for egg hatching was also 15ºC and 45ºC. No eggs hatched at these temperatures, even though some of the eggs started their first six hours of development in prime growing conditions. Both these temperatures would provide protection to honey supers when SHB adults or eggs are present.

At temperatures of 20ºC and 40ºC egg survival is marginal with around 30% of the eggs hatching. At these marginal temperatures there was a wider spread of results, therefore larger standard errors when compared to 25ºC, 30ºC and 35ºC. This is expected where the difference between survival and death is evenly balanced and where minor variations can cause significance variations in the results. The data set suggests that 30ºC is the centre and optimal egg survival temperature with an almost symmetrical decline either side.

When counting the eggs laid it was observed that the proportion of eggs that looked unviable appeared to rise at 35ºC and increase further at 40ºC as compared to the eggs laid at lower temperatures. The unviable eggs generally appeared deflated in appearance however this data was not collected because it was too unreliable to differentiate between viable and unviable eggs.

Egg laying significantly declined with reducing relative humidity levels but a few eggs were laid at 34%, the lowest humidity tested. No threshold temperature to prevent SHB laying eggs was determined. The decline in eggs laid was probably caused by moisture being lost in each egg laid and through desiccation of the SHB, depleting the available moisture in the beetle. If water levels in the body decline to a critical point metabolism slows causing egg production to decrease (Chapman,

1969). If SHB can replenish moisture it may allow them to maintain or start laying eggs again. It is suspected that continued exposure to low humidity levels and no access to any water sources, as in the trial, causes the SHB to reduce egg laying to conserve moisture and prolong life, but continued exposure would result in their desiccation and death.

Unlike the adult and larval life stages, the SHB eggs are immobile and depend on the surrounding conditions being favourable for survival. The eggs are very susceptible to desiccation (Pettis as quoted by (Somerville, 2003)) during their short incubation period. Adult SHB can withstand 34% humidity for 24h at 30ºC but their eggs cannot. Only one egg was found to have hatched out of 2185 eggs exposed to 34% RH. At 46% and 58% RH about 20% and 78% of eggs hatched respectively. Using 44% and 57% RH Pettis (as quoted by (Somerville, 2003)) obtained around 12% and 53% egg hatch respectively. It is suspected that if air movement is increased under low set humidities it would hasten water loss and result in faster desiccation and increase egg losses. Scientific technique may have affected air movement accounting for the variation between the two experiments. Because SHB eggs are susceptible to desiccation in low humidities this could be used effectively to prevent SHB damage within the honey shed. With good air movement the levels of humidity required for SHB management in the honey shed, may not need to be as low as 34%, with Pettis (as quoted by (Somerville, 2003)) suggesting below 50% would be adequate, but this needs further investigation.

The variation in results and therefore the standard errors for 46% and 58% RH were far larger than for 34% and 84% respectively. At 34% and 84% RH the conditions are at either extreme for egg survival regarding humidity so it is quite definitive if eggs hatch or not. When conditions are evenly balanced between life and death the variation in results between replicates increases. The inconsistency of the humidity produced by the honey (~58% RH) also would have contributed to the variation in results.

The impact different temperatures and humidities have on SHB larvae was not examined in this trial, but it would be interesting to know what would be required to kill SHB larvae.

Many commercial beekeepers already have cool rooms installed for pest control management for their honey supers, or warm rooms to warm honey prior to extraction. Results obtained indicate that exposure to 15ºC and 45ºC prevents SHB damage. Honey supers can hold a lot of heat when brought in from the field and if stacked high and tightly together the centre boxes are well insulated by the surroundings supers. To overcome this thermal inertia and to hasten heating or cooling throughout all boxes, they should be stacked to allow good air flow through them. Air movement can be improved with the use of fans.

Hot rooms may be another option for preventing SHB damage however great care needs to be taken in monitoring and achieving an even temperature distribution so that combs do not melt or sag. Sagging and distorting combs is particularly an issue when full of honey because of the additional weight. For practical reasons it is probably safer to avoid this storage technique for combs full of honey. Using combinations of high temperatures (around 40ºC) and low humidities (50% RH), with a lot of air movement, may also provide affective SHB management but needs further investigation. This would lower the risks associated with using high temperatures**.**

The use of cool and warm rooms and humidity controlled rooms will prevent SHB damage but there are advantages and disadvantages between the thresholds, 15ºC, 45ºC and 34% RH. For all, good air movement is essential between and within the supers to obtain and maintain even conditions throughout the room. Using an insect proof room in combination with using 45ºC which kills SHB adults in less than 18 h means the heating could be turned off after adequate exposure. Similarly this will control wax moth (Somerville, 2007). High temperatures also improves honey viscosity allowing easier extracting, but with high temperatures the wax softens and increases the chance of warping and distortion of combs. When heating to 45ºC the high temperatures can increase SHB development and the rate of fermentation spoilage of SHB contaminated frames. To use 45ºC to disinfest material rapid temperature elevation and accurate monitoring is required. SHB adults can survive but not breed at

15ºC so this temperature would need to be maintained throughout storage. This temperature also prevents wax moth damage (Somerville, 2007). The low temperature can increase the honey candying and reduces viscosity making extracting more difficult. However using lower temperatures is more fool proof with far less accurate temperature monitoring required and less urgency to reach the required temperature. Using dehumidifiers to create low humidities for SHB is cheaper in infrastructure, setup and running costs and can allow room temperature manipulation to aid extraction. Air tight rooms help maintain humidity levels which is more crucial in humid regions. The low humidity also helps prevent moisture absorption by uncapped honey.

The approach to SHB management when storing honey supers, full or stickies, will depend on the individuals situation. Consider all possibilities, investigate thoroughly and choose what best suits your situation.

Implications

If beekeepers can keep temperatures in the honey shed either less than or equal to 15ºC or greater than or equal to 45ºC, then SHB will be prevented from egg laying and/or having eggs that survive. Similarly by keeping relative humidity at less than or equal to 34%, SHB eggs would be prevented from hatching.

The implications are that beekeepers need to be made aware of these thresholds and educated in a variety of ways in which they could modify their existing infrastructure and/or management practises to accommodate change. This may include the use of commercially available products such as dehumidifiers, or making structural changes such as building a cool room. The most successful solutions will be those that keep either temperature or relative humidity in the required range, whilst suiting the individual beekeepers' management style.

Recommendations

Further research into the temperature/humidity interaction needs to be undertaken to define the conditions unfavourable to SHB. The role of air movement and exposure time to differing temperature/humidity conditions is another factor which has not been investigated here. Knowing the temperatures, humidities and exposure times required to kill SHB larvae and adults would provide additional tools that could be used in the management of SHB in the honey shed. Further research and development into techniques which rapidly equalise air temperature and relative humidity in a commercial beekeeping storage facility are required to ensure this works practically on a large scale.

Trial 2 – Hive Strength and Susceptibility to Small Hive Beetle

Methodology

Pre–trial hive preparation

Hive preparation started in spring 2007 with stronger colonies being split to get adequate hive numbers for the trial. All colonies were requeened with sister queens of European honey bees between 23 October 2007 and 25 October 2007. All queens were marked with blue paint to enable easy identification and location throughout the trial. Once the new queens had established all colonies were relocated to Bathurst Agricultural Research and Advisory Station on 11 October 2007 where favourable bee breeding conditions allowed build-up of all the colonies. Low SHB numbers at Bathurst minimised the opportunity for *Kodamaea ohmeri* contamination and growth in the hives that may influence the hives attractiveness to SHB when relocated into the SHB area. Hives were inspected mid-January 2008 to ensure the presence of the marked queens. Some queens failed leaving 27 colonies (nine for each treatment) available for the trial. Based on the population within the hives, hives were identified as strong, weak or queenless.

Hives were a mix of both 8 and 10 frame (using 9 frames) Langstroth bee hives. All hives had a brood box, queen excluder and one honey super throughout the trial. The strong hives were left alone with no hive manipulation until just prior to the start of the trial. The hives allocated to the queenless treatment had the queens caught on the 23 January 2008, caged with a few young bees and placed between frames in the middle of the brood box. This was to minimise the colony attempting to replace her as her pheromones were still be present in the hive. The caged queens were kept in the hives for one week, before being removed from the colonies at the beginning of the trial. This allowed adequate time for all honey bee eggs and larvae to develop past the age they could be successfully converted into a queen cell. Any queen cells found were removed.

The hives allocated to the weak treatment were split on the 17 January 2008 to reduce the strength of the hives. Approximately half the bees and frames (brood, pollen and honey) were removed and replaced with dry empty combs. The colonies split from the trial colonies were closed and immediately removed from the area to prevent bees returning to the trial colonies. Any capped honey in the supers of the weak hives was extracted on the 31st January 08.

Trial

All hives were assessed between the 29 January 2008 and 1 February 2008. This included weighing the whole hive using portable digital scales. The hives were then opened and the queen found and caged to prevent injury. To quantify the differences between the hives allocated to each category (strong, weak or queenless) several key indicators of hive health were recorded throughout the trial. These included areas of brood, stored honey and pollen, frame weights, counts of the number of bees returning to the hive over time and weight of extracted honey. For this, each brood frame was removed and the bees shaken off at the hive entrance. Photographs were taken of both sides of all the brood frames for each hive. For each hive the brood frames were collectively weighed. The frames from the super were similarly weighed. SHB numbers in the hives were very low and any found were removed during the examination. The hives were then restored to there original state taking care to return the frames to there original positions and the queen to her colony. Over the next two days, all 27 hives were relocated to the apiary yard at the University of Western Sydney, Hawkesbury campus

(33° 36' 41"S, 150° 44' 45"E) where a high endemic SHB population existed. Hives were randomly placed into two rows in a predominantly shaded area (Figure 10).

Figure 10. Strong, queenless and weak hives in location at UWS.

To minimise the possible influence of hive manipulation on SHB activity, the colonies were only inspected three times during the ten week experimental period - at the start (30 January 2008 to 1 February 2008), mid-way (4 March 2008 to 8 March 2008) and at the end (14 April 2008 to 16 April 2008). As with the pre-treatment inspection, frame weights etc. were recorded at each of these dates. The only difference being, that at the second observation SHB were collected (see SHB collection from hive), counted and returned under the lid of the same colony a minimum of two hours after disturbing the hive. This interval allowed the bees sufficient time to re-establish some order within the colony prior to releasing the SHB. At the end, SHB were collected and counted but were not returned to the colonies.

SBH collection from hive

SHB were collected from each hive with the use of a pooter (a devise used to suck up SHB). To collect the SHB from each colony, hives were smoked, the lids removed and examined. Beetles were aspirated from the lid and top bars of the super. The lid was then placed on the ground and the honey super placed squarely on top. Both the super and the brood box were then left exposed to the light and also smoked for up to a minute to drive the SHB down onto either the bottom board or the lid. The brood box or the honey super was then picked up and placed squarely onto a spare lid. The SHB that were driven down out of the box were collected as quickly as possible to minimise the number escaping. This process was done repeatedly for both hive boxes until no further SHB were seen. During the process of photographing and weighing the frames any SHB seen on the frames were also collected.

All SHB collected from each hive were removed from the pooter and kept separate in ventilated labelled containers that identified the hive from which they came. Later that day the SHB were chilled to facilitate counting before being returned to their hive of origin at least two hours after collection. This gave the bees time to re-establish some order in the colony after the hive manipulation. The SHB were tipped out over the top bars of the super under the hive lid with minimal disturbance to the bees.

Returning bee counts

To quantify bee numbers per hive, and therefore hive condition throughout the trial, the number of bees returning to each colony was counted over 30 second periods on 6 February 2008, 15-16 February 2008, 5 March 2008 and 5 April 2008. Two returning bee counts were taken on 6 February 2008 at 3:15 pm and 4:40 pm with rain before, between and after the counts and 5 April 2008 at 1:25 pm and 3:05 pm on a clear still day. On the 15 and 16 February 2008 three counts, over two days were combined with count one at 2:15 pm on the 15th and the other two at 9:10 am and 9:40 am on the 16th all under cloudy mild conditions. A single count was taken on the 5 March 2008 at 8:10 am. Where more than one count was undertaken in a day the mean was recorded.

Removing honey

During the experiment burr comb from under the lids was removed and weighed on the 5th March 08 and the 18th March 08. Any full frames of ripe honey in the supers were extracted on the 18th March 08. To determine the weight of honey removed all frames were weighed before and after going through the extractor. All frames was returned to the hive of origin shortly after extraction. For each hive the weight of all burr comb and honey extracted was recorded and tallied.

Calculating frame areas

Images of both sides of the brood frames at the start, mid and end of the trial were downloaded to a computer to determine the area of capped worker brood, capped drone brood, capped honey, pollen and the remaining frame area for each hive. The photographs of the brood frames were set to a standard frame dimension of 207 mm by 430 mm using Manifold GIS package. The boundaries for the different area categories were drawn digitally over the photographs as linework using the computer program ArcView GIS 3.x. These lines were then converted to closed polygons and the area for each polygon calculated by the program (Figure 11). Each polygon was then assigned the appropriate class such as honey, pollen etc., again using ArcView 3.x but using a custom script. The sums of each class for all the frames in each hive were computed using ArcGIS 9.x which provided a summary table of the areas in mm^2 ready for analysis.

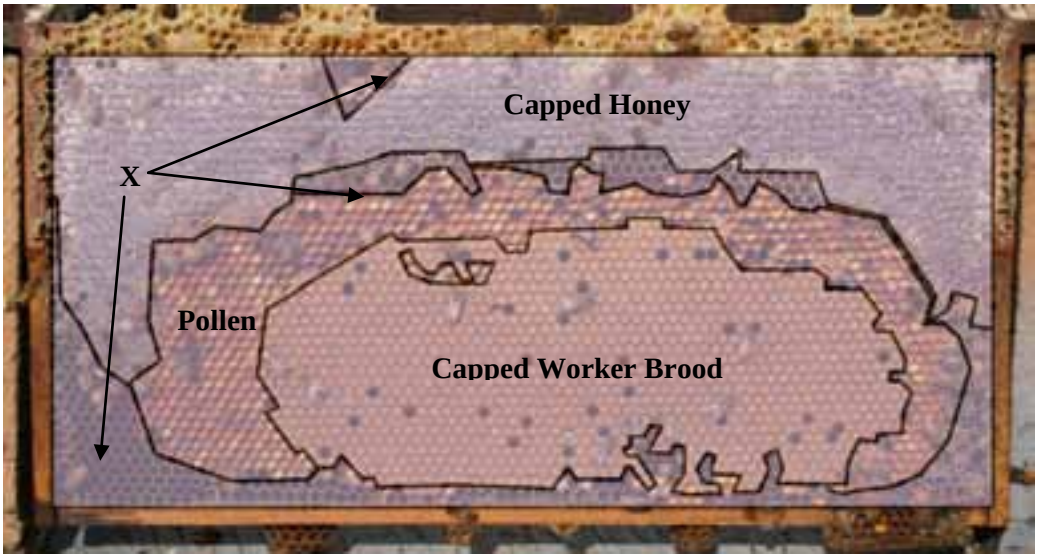

Figure 11 An example of a frame image that has been divided into capped worker brood, capped honey, pollen and other (area x).

Data presentation and analysis

The data was collected primarily to investigate variation between strong, weak and queenless hives.

At the mid point examination, it was noticed that three of the "queenless" colonies had re-queened. Not knowing when the queens established in these colonies, or the genetics of the queens and the lack of continuity between the three, they were removed completely from the data set leaving only six queenless colonies.

Data collected from the photographs gave the areas of worker brood, drone brood, pollen, honey and the remaining frame area (referred to as area x). Each response was analysed using a linear mixed model (LMM) analysis. The fixed effects in the model were *hive condition* (strong, weak and queenless), the *date* (data collected) and the interaction between these two factors. Random effects were hive (fitted as a factor having 24 levels, one for each hive), hive position within the apiary (the two rows of hives ran east to west and were divided up as north east - three hives, north middle - four hives, north west - four hives, south east - four hives, south middle - six hives and south west - three hives) and random error. Homogeneity of the residual variance is assumed.

For bees flying, the LMM analysis was kept simple and included as fixed effects hive condition (strong, queenless and weak), date (treated as a four level factor) and the interaction effect between these two. Random terms in the model were effects for each hive and random error.

For the analysis of total frame weight an adjustment (fixed effect) was included in the model to account for the mix of 8 and 10 frame equipment used in the experiment. Predicted means for hive condition and date combinations were all based on 8 frame hives.

For removed honey a simple analysis of variance was performed and for each hive condition (strong, queenless and weak) a different mean and a different error variance was allowed.

For analysis of SHB number in hives the start date results are excluded because they were zero throughout.

The predicted means and associated standard errors for each trait, based on the analytical model employed, are graphed showing the Least Significant Difference (LSD) ranking using letters. Combinations with a letter in common are not significantly different at a 0.05 level (approximately).

All the mixed model analyses above were performed using either ASReml (Gilmour *et al*., 2006) or under R (R Development Core Team, 2009) using the package *asreml* (Butler, 2009). Within ASReml, tests for significance of fixed effects in the models are based on the methods developed by Kenward and Roger (1997).

Results

Conditions

When looking at the results the influences of changes in seasons and particularly seasonal conditions need to be considered. The start of the trial was very wet with 180.4 mm of rain recorded in the first ten days of February 2008 at the weather station on the University campus (BoM, 2008) resulting in a lot of surface water and saturated soil. The bees came off high-quality breeding conditions at Bathurst with good pollen loads and for the first half of the experiment surplus nectar was available with honey being stored. For the second half conditions deteriorated with limited nectar available for the bees. At the end of the trial during hive examination the bees were robbing the opened colonies.

Frame areas – capped worker brood

There was a significant interaction between *hive condition* and *date* ($F_{4, 42} = 9.7$, $P < 0.001$) on the area of capped worker brood found in the hives (Figure 12). Hence brood differences between hive types (strong, weak and queenless) differed significantly across dates. Similarly, brood differences between dates differed significantly across hive types.

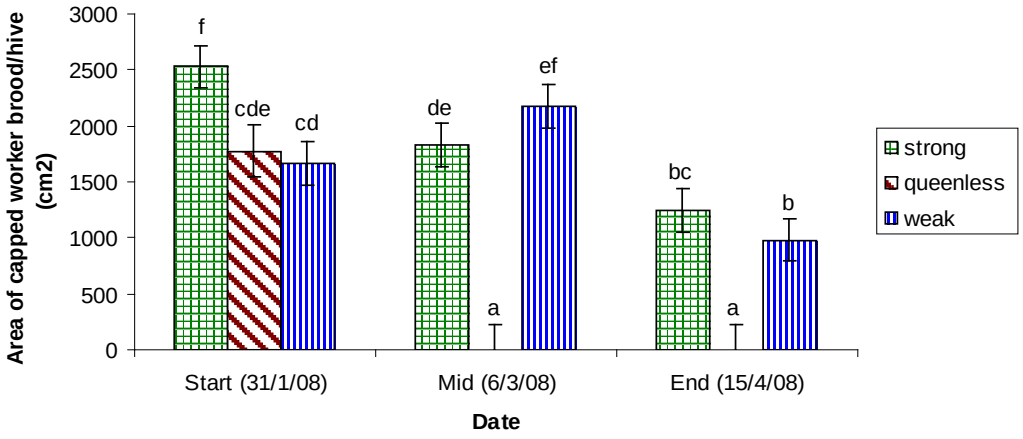

Figure 12. The predicted mean area/hive (cm^2) of capped worker brood in strong, queenless and weak colonies as observed at the start (31 January 2008), mid (6 March 2008) and the end (15 March 2008) of the trial. Bars represent the standard errors of the means and an LSD (0.05) ranking is shown by the letters.

Frame areas – capped drone brood

There was a significant interaction between *hive condition* and *date* ($F_{4, 42} = 6.1$, $P < 0.001$) on the area of capped drone brood found in the hives (Figure 13). Brood differences between hive types (strong, weak and queenless) differed significantly across dates. Brood differences between dates also differed significantly across hive types.

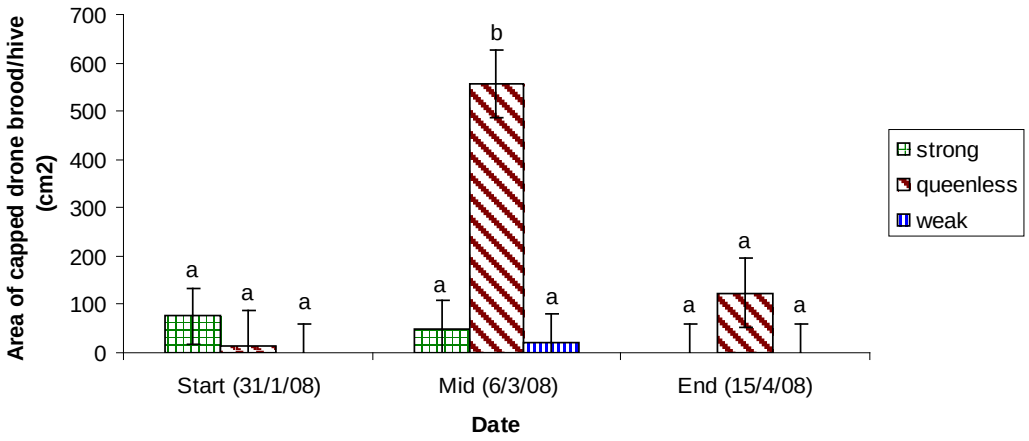

Figure 13. The predicted mean area/hive (cm2) of capped drone brood in the brood box of strong, queenless and weak colonies as observed at the start (31 January 2008), mid (6 March 2008) and the end (15 April 2008) of the trial. Bars represent the standard errors of the means and an LSD (0.05) ranking is shown by the letters.

Frame areas – pollen

There was a significant interaction between *hive condition* and *date* ($F_{4, 42} = 5.8$, $P < 0.001$) on the area of pollen found in the hives (Figure 14). Pollen differences between hive types (strong, weak and queenless) differed significantly across dates. Pollen differences between dates also differed significantly across hive types.

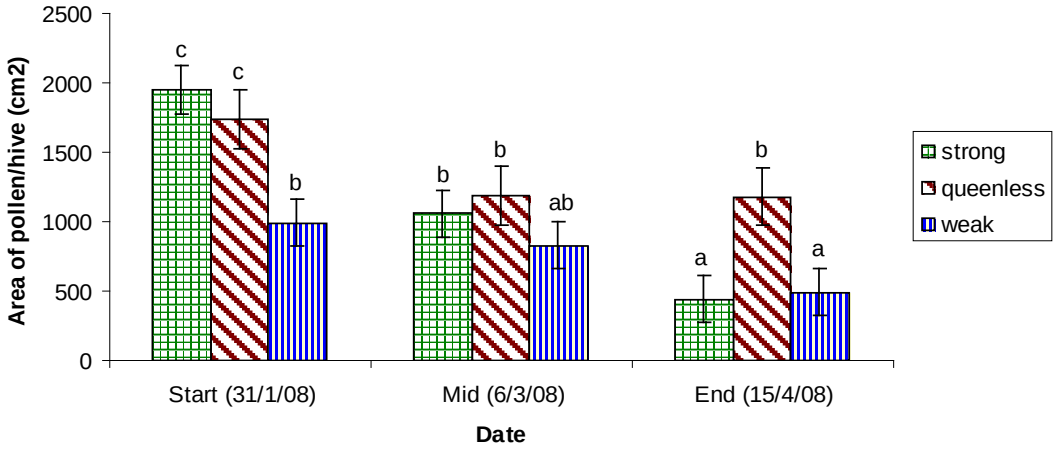

Figure 14. The predicted mean area/hive (cm2) of stored pollen in the brood box of strong, queenless and weak colonies as observed at the start (31 January 2008), mid (6 March 2008) and the end (15 April 2008) of the trial. Bars represent the standard errors of the means and an LSD (0.05) ranking is shown by the letters.

Frame areas – honey

There was a significant interaction between *hive condition* and *date* ($F_{4, 42} = 8.7$, $P < 0.001$) on the area of capped honey found in the hives (Figure 15). Capped honey differences between hive types (strong, weak and queenless) differed significantly across dates. Capped honey differences between dates also differed significantly across hive types.

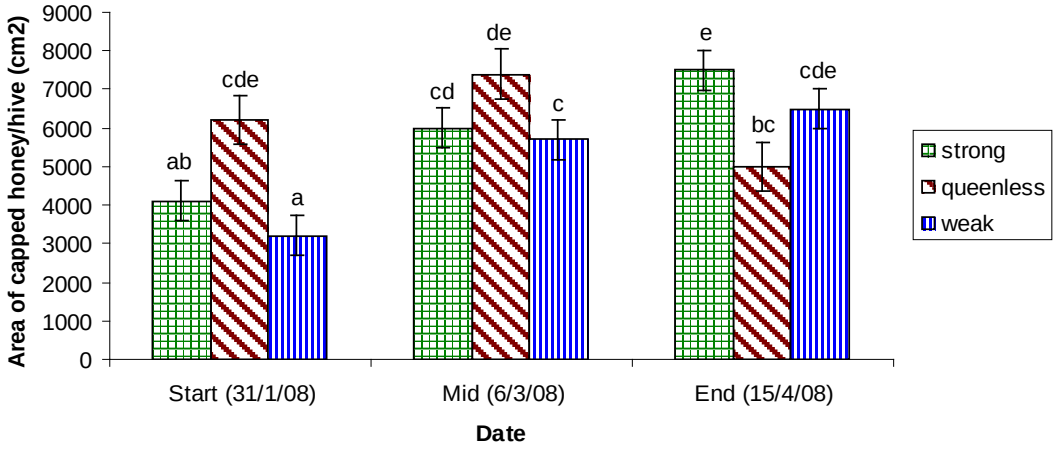

Figure 15. The predicted mean area/hive (cm2) of capped honey in the brood box of strong, queenless and weak colonies as observed at the start (31 January 2008), mid (6 March 2008) and the end (15 April 2008) of the trial. Bars represent the standard errors of the means and an LSD (0.05) ranking is shown by the letters.

Returning bee counts

There was a significant interaction between *hive condition* and *date* ($F_{4, 42} = 10.35$, $P < 0.001$) on the number of bees returning to the hives (Figure 16). Returning bee number differences between hive types (strong, weak and queenless) differed significantly across dates. Returning bee number differences between dates also differed significantly across hive types.

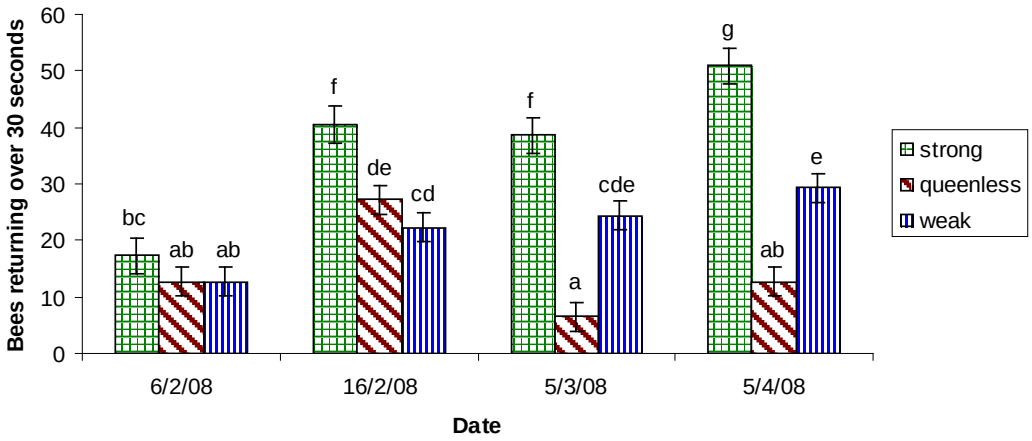

Figure 16. The predicted mean number of bees/hive counted returning into strong, queenless and weak colonies over one to three 30 second periods on the 6 February 2008, 16 February 2008, 5 March 2008 and 5 April 2008. Bars represent the standard errors of the means and an LSD (0.05) ranking is shown by the letters.

Total frame weight

There was no significant difference ($F_{1, 62} = 2.7$, $P > 0.1$) in the total frame weight between the *frame* effect of the 8 or 10 hive equipment, however this term was retained in the model as it would be expected that it would influence the result even though it was not significant.

There was a significant interaction between *hive condition* and *date* ($F_{4, 42} = 5.8$, $P < 0.001$) on the total frame weight of the colonies (Figure 17). Total frame weight differences between hive types (strong, weak and queenless) differed significantly across dates. Total frame weight differences between dates also differed significantly across hive types.

Honey was extracted from those hives with surplus honey and this saw a decline in frame weights between the mid and last observation.

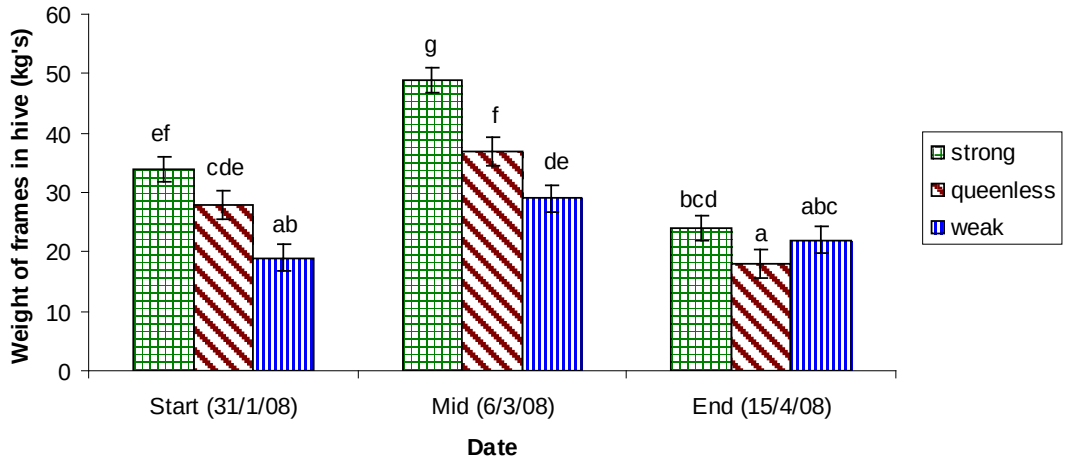

Figure 17. The predicted mean weights (kg) of all frames/hive of strong, queenless and weak colonies as weighed at the start (31 January 2008), mid (6 March 2008) and the end (15 April 2008) of the trial. Bars represent the standard errors of the means and an LSD (0.05) ranking is shown by the letters.

Removed honey

There was a significant effect ($F_{2, 8.3} = 66$, $P < 0.001$) of hive condition on the weight of honey removed from the hives (Figure 18) with the strong hives producing significantly more honey (.~ 3 X) than the queenless and weak hives.

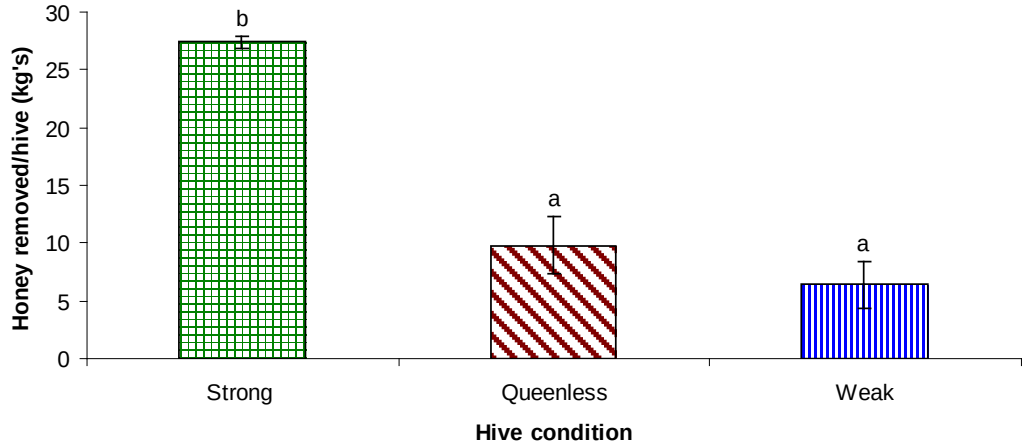

Figure 18. The predicted mean weight (kg) of extracted honey (18 March 2008) plus burr comb (5 March 2008) removed from strong, queenless and weak hives. Bars represent the standard errors of the means and an LSD (0.05) ranking is shown by the letters.

Adult SHB in the hive

There was no significant interaction between *hive condition* and *date* on the number of SHB found in the hives ($P > 0.05$) but both main effects were significant. The *date* effect was highly significant ($F_{1, 23} = 196.4$, $P < 0.001$) whilst the *hive condition* effects were marginally significant ($F_{1, 20} = 4.4$, $P = 0.026$). These effects are shown in Figure 19.

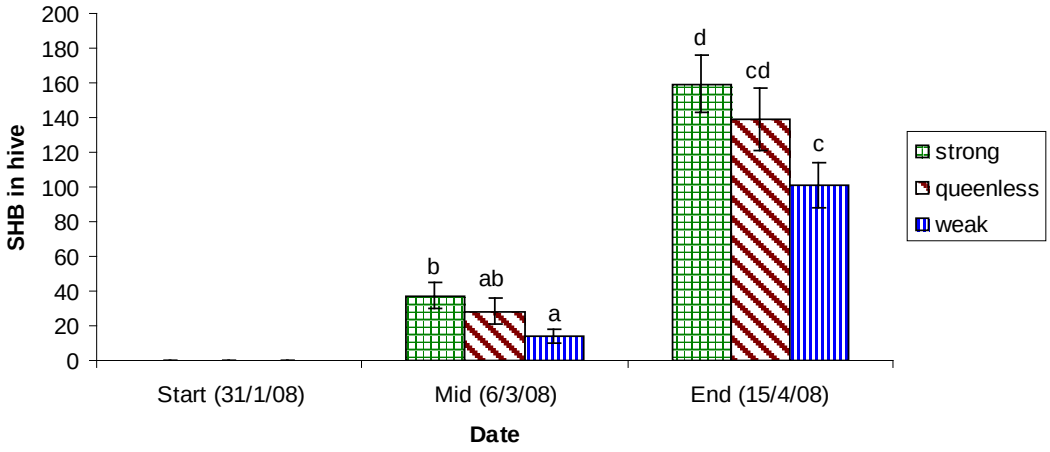

Figure 19. The predicted mean number of small hive beetle/hive counted in strong, queenless and weak colonies at the start (31 January 2008), mid (6 March 2008) and the end (15 April 2008) of the trial. Bars represent the standard errors of the means and an LSD (0.05) ranking is shown by the letters.

SHB larval infestations

Some SHB larvae were found during the mid inspection (5th March 08) between two full frames of honey in the honey super of a strong colony. An area of approximately 7 cm by 7 cm was affected and showing signs of 'sliming'(Figure 20). Only 15 adult SHB were within that colony at the time when the damage was observed.

Figure 20. Area infested and slimed by SHB larvae in a strong colony at the mid point inspection when two honey frames were close preventing bee access.

At the final examination (15th April 08), all six queenless hives had extremely low bee populations, as shown by the counts of returning bees, but all still had bees living and working in the hives. Queenless hives were the only ones to show SHB infestation. Out of the six hives, four had signs of early stages of SHB larval infestation. For three of these hives the SHB larvae were still small, bees were still present and no damage to the combs or fermenting honey was evident. In the other infested hive larval

damage was more advanced with larger larvae present and bubbles in the uncapped honey cells indicating that fermentation was occurring (Figure 21). The few remaining bees had yet to be driven out of the hive by the larval damage.

Figure 21. One of the queenless hives at the end (15 April 2008) infested with SHB larvae and honey bubbling in the cells

Discussion

The results indicated significant differences between the strong, queenless and weak hives throughout the trial period. The difference between the strong and weak hives was large initially but decreases as the weak hives build up throughout the trial. The weak hives initially had less capped worker brood, less pollen and lighter frame weights and for the trial duration had less honey available for extraction and less bees flying for all counts except the first. The differences between the strong and queenless hives were small initially but as the trial progressed the differences increased as the bee numbers declined in the queenless colonies. By the trial's end the weak colonies had built up in strength becoming significantly stronger than the queenless colonies. The three main differences in the queenless colonies at trial end were the reduced number of bees flying, no worker brood and significantly more pollen.

One anomaly in hive condition was the weak and strong hives had no significant difference in bees flying when counted on the 6 February 2008. The count was influenced by the wet weather when done. It may also have been influenced by when the hives were split to create the weak hives. Hive splitting occurred on a hot sunny day and most of the field bees being out remained with the colonies used for the trial. Returning bee counts were affected by weather conditions and the time of day the counts were done, making it difficult to compare between dates. Observations under more favourable foraging conditions may have given a different story.

The strong hives had significantly more SHB at both the mid and end points of observation than the weak hives. However, this did not result in weakened hive health in any observed way. Therefore the strong hives proved to be resilient to the SHB. By the end of the trial there was no evidence of SHB larvae or sliming in the strong hives.

One strong hive had SHB larvae detected at the mid point of the trial after two bulging frames of honey comb were placed together preventing bee access between the frames. Once the frames were separated, the bees removed the SHB larvae and repaired any damage leaving no signs of SHB damage and no larvae were found again. This demonstrated the ability of the SHB, even if present in low numbers (15), to take advantage of situations where bee access is denied in the hive, regardless of hive strength.

Strong hives have more bees, bee brood, food reserves and bee activity resulting in the release of greater amounts of air volatiles (smells) as compared to weaker hives. It is suspected that the stronger hive odours result in more SHB being drawn to the stronger hives.

The queenless colonies had less SHB than the strong hives at both the mid and end point inspections, but not significantly so. Other studies in this area have found similar results (Spiewok *et al.*, 2008) and this is contrary to the commonly held view by beekeepers that queenless hives are the most attractive to SHB attack. The queenless colonies had developed large pollen reserves with no bee larvae to feed and it is possible that, with limited bees to keep guard, these pollen reserves became contaminated by SHB carrying the yeast *K. ohmeri.* The growth of the yeast and the subsequent production of the chemical isopentyl acetate (IPA) (Torto *et al.*, 2005) may then have been responsible for attracting more SHB to the queenless hives (Torto *et al.*, 2007). With the demise in strength of the queenless colonies it could be considered the SHB numbers may also decline however the *K. ohmeri* contamination and release of IPA may have countered the decline in hive strength resulting in the queenless colony continuing to be as attractive as the strong colonies.

Being queenless caused the hive populations to decrease over time and this left the colonies bereft of bees to defend the colony. Four of the six queenless colonies had some early stages of SHB larval activity after 11 weeks of bee decline. It is likely that the larvae infested colonies would have succumbed within a week with the remaining bees abandoning the hive. Therefore as bee numbers decline the hive becomes highly susceptible to the ravages of an SHB infestation and would have eventually collapsed as a direct result.

The weak hives had the smallest SHB populations at both the mid and final points of observation, significantly less than the strong hives. Unfortunately SHB numbers in the environment were low at the beginning of the trial when the weak hives were at their weakest and most vulnerable to SHB. It would have been preferable to see the impact of high SHB population pressure on them at this time. By the time SHB numbers in the environment increased the weak hives had rebuilt themselves to a certain extent, sufficient to overcome the stresses caused by SHB infestation. No larvae were detected in any of the weak hives during the trial.

Data gathered from this trial provided evidence that SHB are more attracted to strong hives than weak, and that queenless hives were attractive to SHB but no more than strong hives and no less than weak hives. It was also shown that inadequate bee numbers, as found in the queenless hives over time, can expose the colony to the ravages of SHB, to the point of hive collapse.

Implications

The results show that bee colonies become vulnerable to SHB larval damage once there are insufficient bees present to protect the colony or when bees are prevented from accessing the frames. The implications are that beekeepers need to act as soon as they identify a problem of this nature.

By being proactive and removing, joining or compressing the high risk colonies as soon as possible, colony losses to SHB can be reduced. This will allow time and monetary saving for the beekeeper. It also helps curtail the SHB population therefore reducing the SHB pressure on remaining colonies.

Recommendations

What makes one hive more attractive to SHB than the hive next door is still not well understood despite a lot of previous and continuing research. Determining what draws SHB to a hive still has the potential to be developed into an outside lure that is more attractive than the hive to capture SHB in and around the apiary.

Trial 3 – Movement of Small Hive Beetle In and Out of the Hive

Methodology

Surveillance recording

Four 2 deck hives of European honey bees were relocated from Bathurst to the apiary yard at the University of Western Sydney, Hawkesbury campus (33° 36' 41"S, 150° 44' 45"E) on the 1 February 2008. This area has a high endemic population of SHB. The hives were placed close to the honey extraction shed to enable access to power and shelter for the surveillance recording equipment. The four hives where placed on concrete which allowed easy detection and identification of the SHB approaching the hives. Two hives were against the wall of the extraction shed about one metre above ground level and the other two were three metres out from the wall at ground level (see Figure 22).

Figure 22. The four hives with surveillance cameras attached and recording. The top right hive was Hive 1 and proceeding anti-clockwise Hive 2 etc. as numbered.

An XPOSE 4 Channel DVR/Camera Surveillance Kit (QV 3070) distributed by Electus Distribution Pty. Ltd. was used to monitor SHB movement to and from the four hives. The four cameras in the kit were waterproof and had infra-red night viewing capabilities. The digital recorder had a 250 Giga byte hard drive that enabled many days' recording to be stored before data was over written. Fifteen metre long cables allowed cameras to be setup within that distance of the recorder. Each camera was attached to a wooden structure that was strapped against the lid of the hive by an M-lock strap. Four litre plastic ice cream containers were placed over the cameras to protect them from the weather and minimise glare. Cameras were adjusted during set up to ensure good coverage of and around the hive entrances. All four images relayed by the cameras could be viewed at once or individually on a

monitor before and during recording. On the screen was displayed the date and time of the recording which allowed the time to be assigned to each SHB movement.

The cameras were set up monthly, recording the hive entrances over a 24 h period. Fourteen recorded observation periods were conducted around the start of every month, between the 25 February 2008 and 4 May 2009, except for May 2008 which was missed. Following a recording session the cameras were removed and stored until the following month. On completion of the 24 h surveillance recording, all four hives were inspected and adult SHB collected, counted and returned to the respective hive.

Hive management

Hives were kept in the one location throughout the trial. Additional honey supers were added on the 5 November 2008 to Hives 1, 2 and 3 to a maximum of two supers (three boxes/hive). Hive 4 was found to be queenless on the 5 November 2008 and was successfully re-queened on the same day. Honey was extracted on three occasions during the trial on the 4 April 2008, 10 November 2008 and 2 January 2009. Clinical symptoms of American foul brood (AFB) disease was identified in Hive 2 on 8 April 2009. The hive was assessed as having adequate strength to protect and maintain its integrity until the following month's recording, so was kept in the trial and re-assessed the following month. On the 5 May 2009 Hive 2 was in the early stages of breakdown with SHB larvae present. The remaining three hives were also found with AFB symptoms so the trial was finished one month earlier than planned to prevent the possible spread of AFB.

SHB collection from hives

After each 24h video surveillance recording was completed the hives were individually opened and all SHB collected, counted and returned as per **Method in trial 2 'SHB collection from hive'**.

On all but three occasions, the SHB were returned to the colonies of origin. On the 10 February 2009, 8 April 2009 and 5 May 2009, the SHB were removed and not returned to the hives. This was done because of very high numbers of SHB and the fear that returning the SHB may cause the hives to succumb to SHB damage.

Surveillance footage examination

The recorded surveillance footage was reviewed on a television at a later date. Footage was examined closely and when a SHB was seen either entering or leaving the hive entrance the time, date and movement direction (in or out) was recorded. Most of the footage was examined at four times normal speed and slowed and reversed when a beetle was seen. During times of frequent SHB observations footage was examined at normal speed. The SHB movement times were then correlated to weather data collected at 30 min intervals, from a Campbell scientific weather station located approximately 300 m from the trial location.

Data presentation and analysis

To examine the time of day of SHB movements, the 24 h day was broken into 12 two hour increments with nightfall taken as zero hours. Nightfall was deemed to be 15 min after sunset for the nearby Richmond Railway Station as determined by Geoscience Australia (www.ga.gov.au/geodesy/astro/sunrise.jsp). The two hours after nightfall were called 0-2 followed by 2-4, 4-6, ….., 10-12. The hours before nightfall being 2-0, 4-2, ….., 12-10.

The counts for SHB entering the hives at different times during the 24 h of surveillance were analysed using a generalised linear mixed model (GLMM) assuming a Poisson distribution with a log link, with the model for the log (mean count) given by

Log(mean count) = baseline + hive + time + hive:time ***+ spl(time) + hive:spl(time) + ftime + hive:ftime + fmonth + hive:fmonth***

The terms in **bold italic** above were fitted as random effects. The model includes a spline model over time, using the midpoints of the time intervals as the "time" covariate. This is allowed to vary across hives, and includes random variation about these smooth spline trends due to *ftime* (a factor version of time with 12 levels); an interaction of *hive* and *ftime*; *fmonth* (a factor with a unique level for each observation month, 14 in total) and an interaction of *hive* and *fmonth*.

A standard linear model was used to analysis the number of SHB within each of the four monitored hives. The data was first log (base e) transformed. The model had as terms a trend in month that was modelled as a spline, a seasonal trend with a 12 month cycle, and specific seasonal effects. The spline trend with month was allowed to differ across hives. Deviations associated with each month were also included as random effects.

Results

From the recorded surveillance and from watching the usual approach taken by SHB entering a colony was to fly into the vicinity of the hive and attempt to land within close proximity of the hive entrance. They usually landed within a 30 cm radius of the hive entrance and then walked fairly directly towards the entrance. Very few SHB flew directly into the hive entrances or onto the hives' landing boards. SHB did not always immediately enter the hive entrance, often going under the hive for varying lengths of time. Guard bees often attempted to prevent their entry resulting in the SHB retreating usually under the hive to try again later.

Very few SHB entering the hives after nightfall had flown in after dark. Most had been in the vicinity of the hive (mainly under the hive) and then had walked in after night fall.

SHB leaving the hive would walk out of the hive entrance onto the landing board and almost immediately fly off.

On some of the hotter recording days during the summer the hives developed bee beards (bees hanging around the entrance) making it difficult to observe SHB movements. These bee beards generally were not too obstructive to viewing during the day but got worse late in the afternoon and lasted well into the night, receding back into the hive a couple of hours before sunrise.

Variation in monthly recordings of SHB movements

For the 14 periods of 24 h surveillance 453 SHB were observed entering the four hives, and 34 leaving. Over the trial period Hives 1 and 2 combined had 291 SHB observed entering them as compared to 162 for Hives 3 and 4.

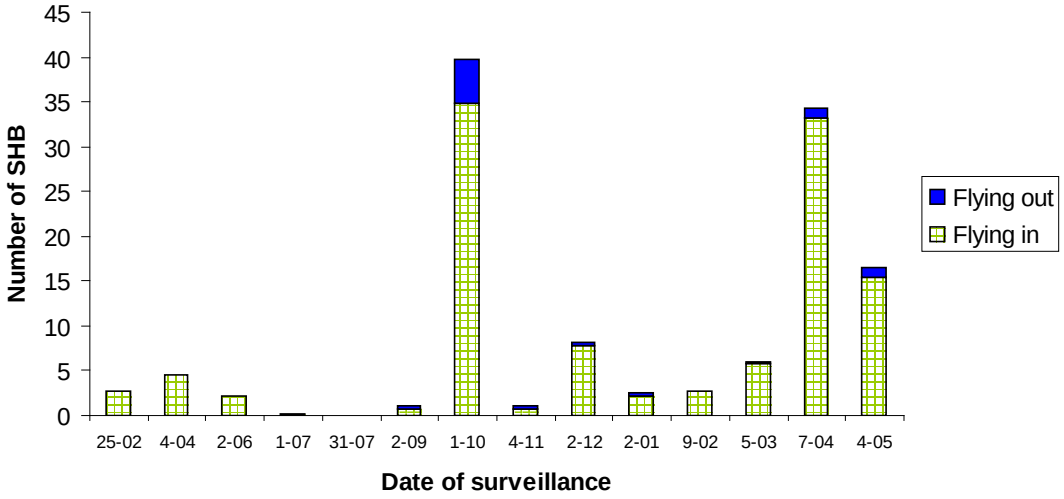

Figure 23. Mean number of SHB entering/leaving the entrances of each hive over a 24 h period of monitoring for 14 observation periods from 25 February 2008 to 4 May 2009.

Time of day SHB entered and left the hives

Some 78% of SHB entered the hives in the 6 h around nightfall (4-2, 2-0 and 0-2) with the majority (51%) entering in the last two hours (2-0).

Fitting the GLMM to model SHB entry numbers indicated that the terms *ftime* and *hive:ftime* were not significant and that the trend in time for the mean number of SHB entering a hive is smooth and adequately captured via the spline models. This smooth trend was significant at $P < 0.05$. There was significant variation across months of observations and to a lesser extent, variation across hives within months after adjusting for the trend in time. The predictive model indicates rapid increases in entries shortly before nightfall (dusk) and a subsequent rapid decline immediately after dark.

For the periods 4-2 and 2-0 h when there was most SHB flight activity a Least Significant Deviation (LSD) ranking showed significantly more SHB entering Hives 1 and 2 (against the wall) as compared to the Hives 3 and 4.

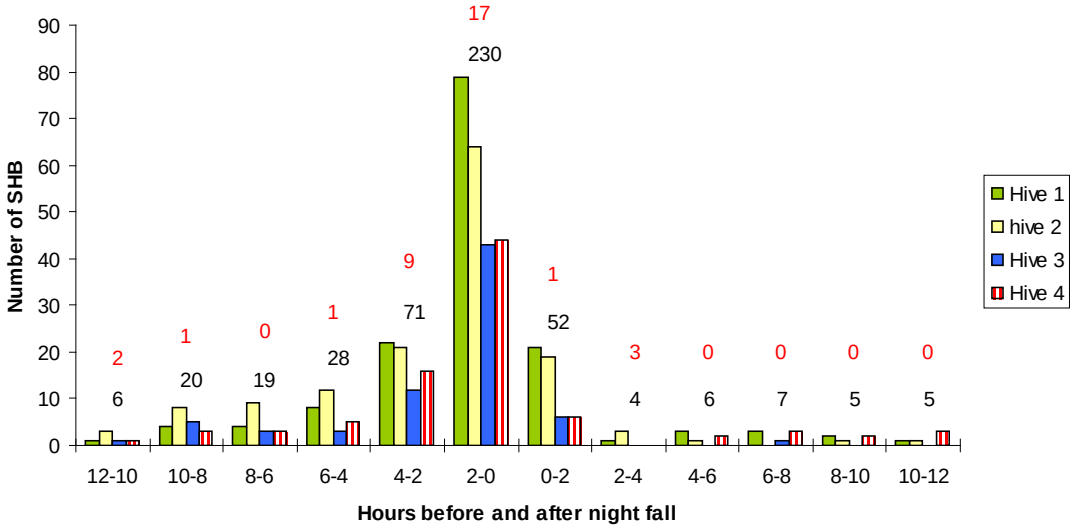

Figure 24. **Total number of SHB entering each of the four hives throughout the 24 h observation over the 14 observations from 25 February 2008 to 4 May 2009, with nightfall being 0 h. For each period, the bottom numbers (in black) above each group of columns are the total SHB entering the four hives. The top number (in red) is the total number of SHB leaving the hive.**

The influence of temperature on SHB movements

80% of SHB movements were observed while air temperature was between 20ºC and 28ºC.

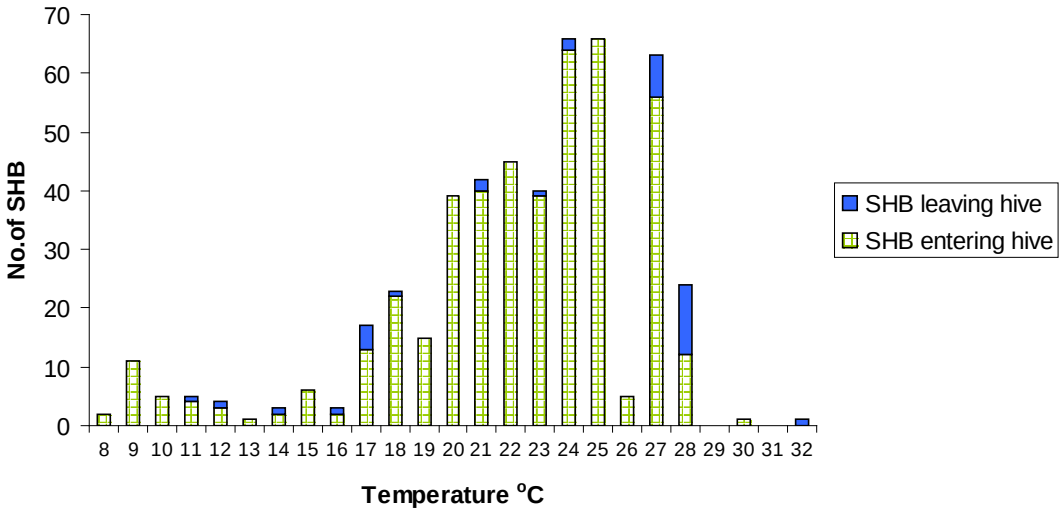

Figure 25. **The number of SHB observed entering or leaving the four hives at different air temperatures.**

The influence of humidity on SHB movements

Of the SHB observed entering hives at 20 to 24% RH, 99 out of the 100 occurred during the October 08 surveillance. For the majority of SHB movements at other RH's there was a much broader spread of dates on which the movements were observed.

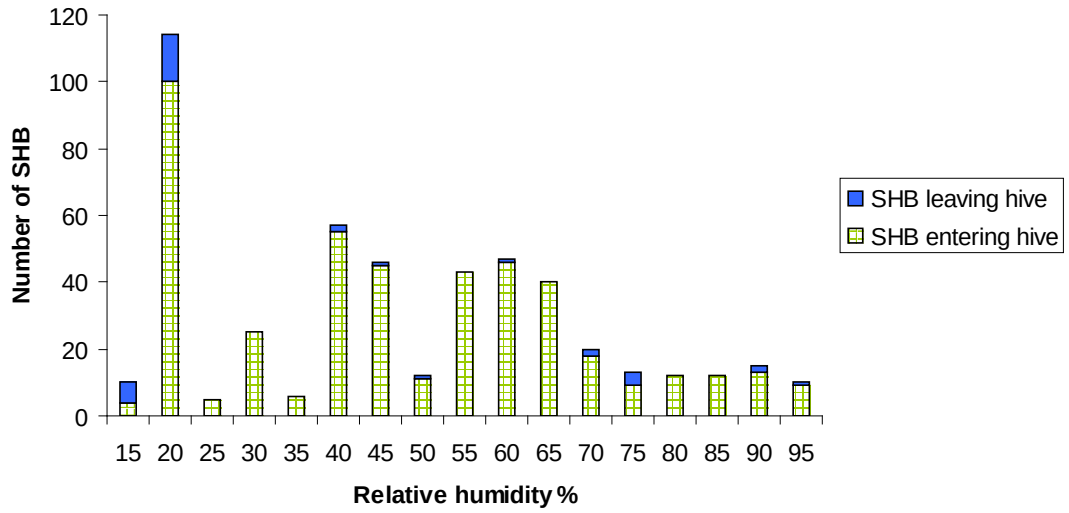

Figure 26. The number of SHB observed entering or leaving the four hives at different relative humidities. The RH for 15% includes 15 to 19%, 20% includes 20 to 24%, etc.

SHB numbers within the hives

The standard linear model looking at SHB numbers within the hives indicated a significant seasonal cycle in addition to significant random monthly deviations. Further, there were significantly different trends over time (months) across hives with the two hives located against the wall (Hives 1 and 2) having higher numbers of SHB than the two front hives (Hives 3 and 4) at each monthly recording for the majority of observations (11/14) of the experiment.

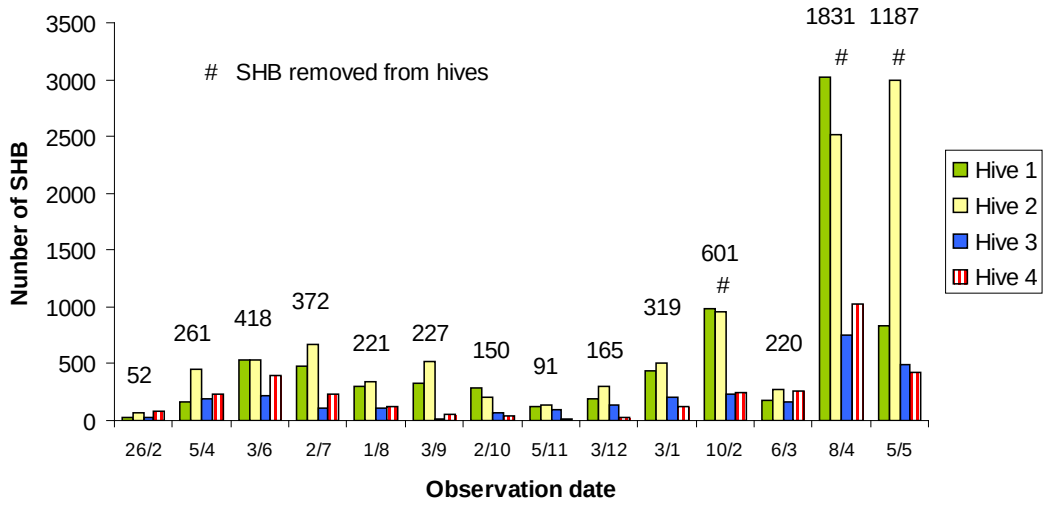

Figure 27. Number of SHB collected, counted and returned within each hive after 24 h surveillance. Numbers above each set of columns is the mean number of SHB per hive (# indicates the occasions the SHB were not returned to the hives to prevent SHB induced hive collapse).

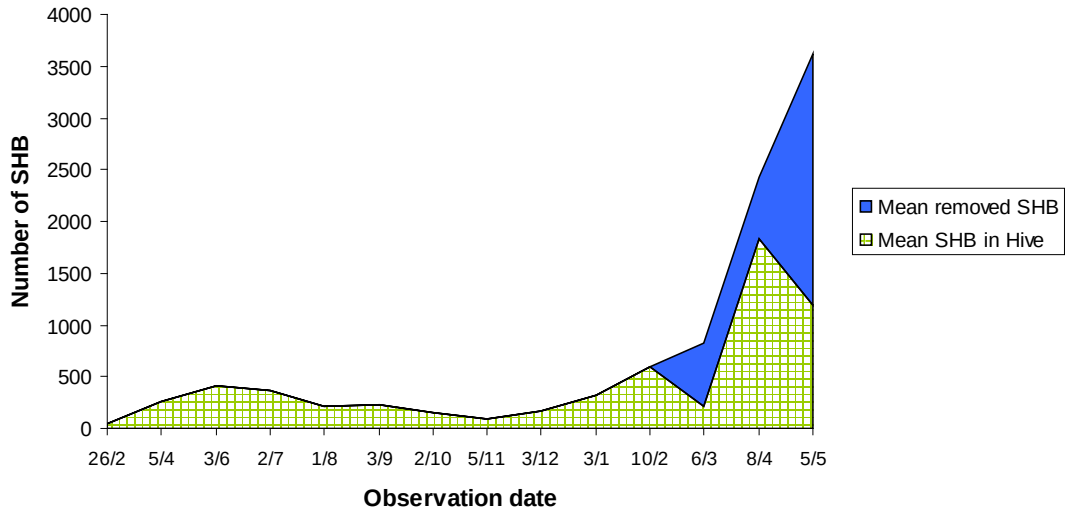

Figure 28. Mean number of SHB collected, counted and returned within each hive after 24 h surveillance from 26 February 2008 to 5 May 2009. The blue area shows the cumulative mean number of the SHB that were not returned to the hives.

Discussion

In the trial far more SHB were observed entering the hives than leaving them at all recording times. For the months of November through to June this resulted in a net increase in SHB numbers within the hives. Therefore their ability to enter and survive within the hive during these months outweighed their removal through exiting or death. In the months of June through to November the opposite was observed, with a net decrease in SHB numbers within the hive recorded. With greater numbers still entering than exiting the decrease in the number of SHB within the hive was most likely due to death. SHB have been observed to live for around six months in favourable laboratory conditions (Ellis, 2003a; Lundie, 1940), which helps explain the population decline from June through to November when cooler temperatures limit SHB breeding and emergence.

SHB have not been collected and positively identified from non bee related sources (pers. comm., Meridyn Davidson NSW I&I, Hamish Lamb Qld DIPWE and Joe Riordon Vic DPI in 2010), and only a small proportion of SHB were observed leaving hives therefore recruitment of newly emerged beetles was probably the main source of SHB in the hives. The time taken from oviposition to emergence of SHB has been studied for a range of temperatures, 34ºC/ 23 days, 24 to 28ºC/39 days (de Guzman and Frake, 2007), 30ºC/32 days (Schmolke, 1974), 18 to 25ºC/41 days (Muerrle and Neumann, 2004) and 17 to 24ºC/49 days (Neumann *et al.*, 2001b). In this trial and trial four SHB population numbers followed a sinusoidal curve, with increasing population numbers occurring between November and June due largely to favourable breeding conditions. If SHB movements are mainly influenced by emergence levels it could be expected the highest levels of infestation of SHB to occur from December to June.

To a large extent movements recorded in this trial correlated well with this pattern. The only exception was a large spike in movements recorded at the beginning of the month of October, larger than at any other time of the year. If these results are a true reflection of movement patterns then ‘where did the SHB come from’?

The October spike of SHB movements also had the highest observed number of SHB leaving the hives with one in ever eight beetles departing and this is possibly an under estimate due to the speed SHB exit the hives. However the high number of SHB had to be coming from somewhere and with little

evidence of SHB adults surviving on alternate hosts in the environment, it is suspected the bulk of SHB entering were recently emerging adults.

Temperatures prior to October were regularly below 17ºC, limiting the opportunity for SHB to complete a full reproductive cycle after winter. One explanation could be some form of dormancy/diapause. Diapause in developing insects can result in the synchronising of adult emergence (Chapman, 1969). Interestingly Pettis and Shimanuki (2000) found both larval and pupal stages of SHB in the soil during February (winter) in Florida perhaps in a state of dormancy. If SHB can overwinter in the ground, emerging post winter, they may be better positioned to survive well into the warmer summer months and take advantage of improved conditions to reproduce.

The peak in SHB movements in October did not correspond with an increase in the total number of SHB found in the four hives suggesting it may have been a short lived spike of SHB movements. Only observing one day a month limits what can be ascertained from this peak in the data. Which raises the question of 'where did they go'? Was the natural attrition rate greater than the SHB influx? This time of year is approximately five to six months after the peak population period of the SHB in the hive.

The majority of SHB movements occurred in the temperature range 17 to 28ºC, with movements observed as low as 8ºC and as high as 32ºC. The temperatures at which most movements occurred strongly correlate with the months of October through to May. As the temperature during the hive surveillance never exceeded 32ºC (see Appendix 1 for maximum and minimum temperatures and humidities for all 24h surveillance periods) during the trial, it provided no opportunity to observe SHB movements at greater than 32ºC. With temperatures rarely above 28ºC during the surveillance period the number of SHB movements was biased towards the more commonly experienced lower temperatures. So from the results it is difficult to determine if temperatures greater than 28ºC affect SHB movements. The majority of the movements at less than 17ºC were SHB that had flown into the hive vicinity earlier, moved under the hives and reappearing once the temperatures had fallen later.

When the temperature was within the range of 17 to 28ºC the time of day was the main influence on SHB movements. The majority of SHB movements occurred in the four hours prior to, and the two hours after nightfall. Although the flight times were not recorded the observation was made that very few flights occurred after nightfall and those that did were very early in the evening.

There were no clear trends in SHB movements observed over a broad range of RH's.

There was an unusual spike in SHB movements at 20 to 24% RH with 99% of these movements occurring during the October 08 surveillance. The low humidity level (20 to 24%) coincided with the busiest flight time, the two hours prior to nightfall, during the busiest observation period.

The hives that were positioned against the wall had greater numbers of SHB movements and larger populations of SHB. Some factors that may have contributed to the variation include, higher position, against a wall, located behind the other two colonies and shaded for longer during the day. The wall provided a barrier which limited the SHB from moving beyond these two hives. Hives 1 and 2 were in shade for longer each day because of their proximity to the building. There are suggestions that hives placed in the full sun will have less SHB than those in shade (Cannon, 2006). De Guzman et al.(de Guzman *et al.,* 2010) noted that the number of SHB in two apiaries in close proximity, where fewer in the one in direct sunshine compared to the tree shaded apiary. Similarly, SHB traps located in shade caught more SHB than those exposed to full sunshine (Arbogast *et al.,* 2007), however Ellis and Delaplane (2006) found the initial dispersal of SHB within an apiary was not influenced by shade.

Hive strengths were fairly similar between hives except for a few months when hive 4 was found to be queenless in November 08. This left this hive weaker than the others for a few months. This coincided with it having the lowest number of SHB relative to the other hives, possibly as a result of being weaker. Near the end of the trial (April 09) clinical symptoms of American foulbrood disease (AFB) in Hive 2 expanded causing a decline in both bees and hive strength. The resulting number of SHB

found in the Hive 2 on the last recording date was over three times greater than for the other hives. Such a large difference implies something was happening to that hive that was attracting the SHB. Hive 2 was starting to breakdown with SHB larval damage. Despite declining bee numbers, Hive 2 was still moderately populated with bees in May 2009. However the extremely high adult SHB population within the colony must have been unmanageable for the bees present resulting in the SHB larval infestation. Perhaps the large number of beetles was a consequence of the AFB infection. Possible reasons why SHB were drawn to Hive 2 include the decline in hive strength, the early stages of SHB larval damage causing contamination and growth in pollen and honey of the SHB carried yeast *K. ohmeri*, or the perhaps the AFB infection and resulting bee larval break down.

Implications

Understanding the fluctuations and cycles of the SHB hive population over the year is important when considering the timing and type of control practises used. The implications are that beekeepers can target their control practises more effectively with regards to hive health and stability and SHB population size and vulnerability.

The possibility of SHB in stages of development surviving through winter by entering a dormancy period, emerging the following spring and allowing replenishment to the declining/aging SHB population, could explain why SHB are a much greater problem in areas with milder climates as compared to cooler areas.

Recommendations

An interesting observation made during the course of this trial suggested SHB have the ability to overwinter in a non-adult stage. This possibility needs to be further investigated as it may provide partial explanation as to why SHB survive so well under certain conditions. It may also allow for the development of targeted control strategies aimed at this aspect of their life-cycle.

Trial 4 – Proximity of Small Hive Beetle Outside of the Hive

Methodology

To capture SHB on the ground in the vicinity under and around where a bee hive had been sitting, six rectangular (121 cm x 100 cm) trapping enclosures were constructed from lengths of steal plate 130 mm×5 mm. Aluminium flyscreen was attached to the top of the enclosures and sealed to prevent SHB from escaping (see Figure 29). The bottom edge of the steel enclosure was sharpened to allow for easier penetration of the steel frame into the soil to prevent any SHB from escaping.

A lure was placed within the enclosure to attract the trapped SHB. The lures comprised of opaque fishing tackle boxes, as SHB have an aversion to light (Somerville, 2003; Homewood, 2010), with four 7 mm diameter holes drilled into the four corners of the base of the box. A piece of comb (approx. 50 mm × 50 mm) containing capped and uncapped brood and pollen was cut from a freshly removed frame, put into the tackle box with a piece of moist paper towel and the lid closed (Figure 30).

Figure 29. Shows the screened enclosure placed over where the hive was moved from. A lure is within the enclosure and a shovel is in the background. A hive lid is yet to be place on the screen over the lure.

A few different container types and attractants for the lure were tried. The fresh comb was found to be the most successful at attracting the SHB. To test the efficacy of the lures the enclosures were set up and 100 SHB were released within the enclosure. SHB were recaptured, removed and counted over the proceeding week with lures checked every couple of days. This was done on seven occasions with a mean recapture rate of 91.5%.

The trapping enclosures and the lures were placed in the location of six bee hives at the apiary yard of the Hawkesbury campus of the University of Western Sydney. This was replicated on fifteen occasions between February 2008 and May 2009, usually monthly. Different bee colonies were used throughout the trial. All were two decker 10 frame Langstroth hives that had been in the apiary for many months before use. Hive locations varied from receiving full sun to dominant shade.

Figure 30. A lure – a piece of freshly removed comb containing brood and pollen placed within a tackle box containing four holes in the base. Still requires a piece of moist paper towel and the lid closed.

Before setting up the enclosures each of the hives were tilted in location to examine and collect any SHB under the hive either on the soil, in the debris/grass or on the base of the hive. All SHB seen were collected using a pooter, counted and included in the SHB outside hive tally. The hives were then moved about one metre forward in the direction the entrances faced, keeping the same alignment to minimise confusion for the returning bees. The lure was then placed, holes facing down, where the hive entrance had been prior to moving. The enclosure was immediately placed squarely and evenly over where the hive had been removed from. Once the enclosure was in position a flat spade was used to cut into the soil, roots and grass around its edge to allow easier embedding of the enclosure into the soil. A sledge hammer was then used to drive the enclosure frame about 5 cm into the soil to prevent the SHB escaping. Once in position a hive lid was placed onto the screen over the lure for protection from the sun and rain.

Once all six enclosures and lures were in place the repositioned hives where opened and all SHB collected and counted as per **Method in trial 2 'SHB collection from hive'**. The SHB where then either returned to the hive of origin, removed completely or released within 15 metres of the front of the hives they had been collected from.

The lures within the enclosures were checked every one to three days over the following week and any SHB found within the lure and enclosure was included in the SHB outside hive tally. After each inspection the enclosure was returned to how it was. Once the SHB collection period was over the enclosures where removed, lure containers emptied and washed out and the hives returned to their original positions ready for the next monitoring.

Data presentation and analysis

Since the same hives were not consistently used across the trial, and taking into account the period between observations, the data are treated as independent hives across observation periods.

To analyse the seasonal variation in *SHB total* for the hives, where *SHB total* is the number of adult SHB both inside and outside the hive, a quantitative variable *month* is first calculated with month equal 1 for Feb 2008, 2 for Mar 2008, ..., 16 for May 2009. *SHB total* data, log (base e) transformed, was analysed using a linear mixed model analysis. Fixed effects in the model are a sinusoidal trend over month (with a yearly cycle), a linear trend with month and distinct effects associated with each season. Random effects are deviations associated with each month and random error.

The proportion of SHB found outside of the hives was expressed as a percentage where

% SHB out* = 100 × *SHB out* / *SHB total. The ***SHB out*** numbers are modelled as binomial (possibly over-dispersed) with N (population size) equal ***SHB total***. The expected proportion outside, on the logistic scale, for each observation is modelled as a linear combination of different effects. These effects initially included as fixed effects a sinusoidal trend over month (with a yearly cycle), a linear trend with month and distinct effects associated with each season. Also included, as random effects, are deviations associated with each month.

Results

On two occasions (the 1 October 2008 and 4 November 2008) one of the six hives was found, post data collection, to contain a SHB control device and hence data from these two hives were removed from subsequent analyses. For these two hives there were found, four and zero SHB within and zero and six SHB outside the hive respectively.

When examining under the hives prior to moving them forward and setting the enclosures, it was generally found the majority of SHB collected were in close proximity to the hive entrances. Often they were under the front cleat or deep in the grass/litter adjacent to the entrance.

The largest number of SHB found outside a hive was 148 in February 2009. The highest proportion of SHB found outside a hive, as compared to the total SHB (excluding the hive with a SHB control device) was (92/132) 70%. On five occasions out of 82 more SHB were found outside the hive as compared to inside the hive.

For the observation of 8 April 2009 five of the six hives were predominantly shaded for the day while the remaining hive (entrance facing north) was in full sun. The number of SHB found outside this hive was 20 as compared to one SHB found outside the five shaded hives. Similarly on the 4 May 2009 four of the six hives were mainly shaded with two in full sun but facing opposite directions, one north and one south. Again only one SHB was collected from outside the shaded hives, one was outside the hive in full sun facing south and 21 SHB were collected from the hive in full sun facing north.

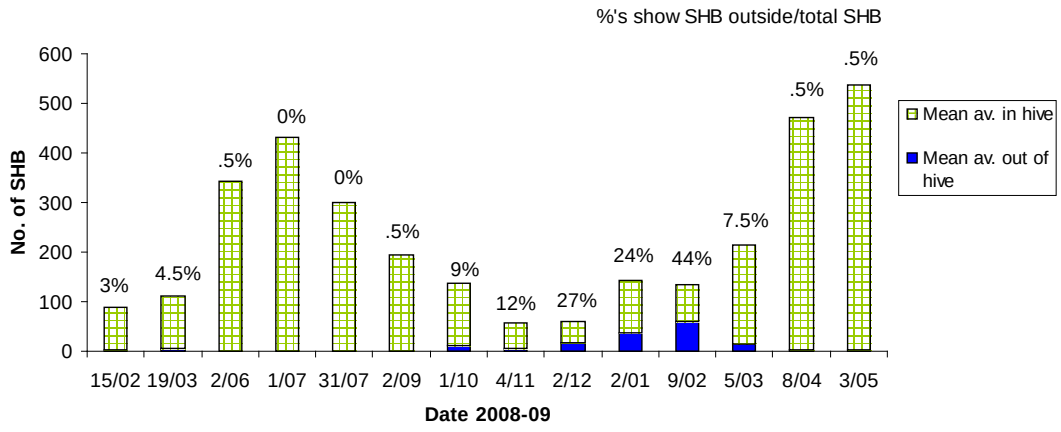

Figure 31. The total mean SHB for each observation from 15 February 2008 to 4 May 2008 with SHB counts for inside and outside the hive. Percentages above columns give the proportion of SHB found outside relative to the total SHB.

There was a significant sinusoidal trend ($P < 0.001$) and a significant linear trend ($P < 0.001$) over time, increasing with months. The distinct season effects are not significant after removing the sinusoidal and linear trend over months. The deviations for monthly averages about the trend (linear and sinusoidal) were not significant.

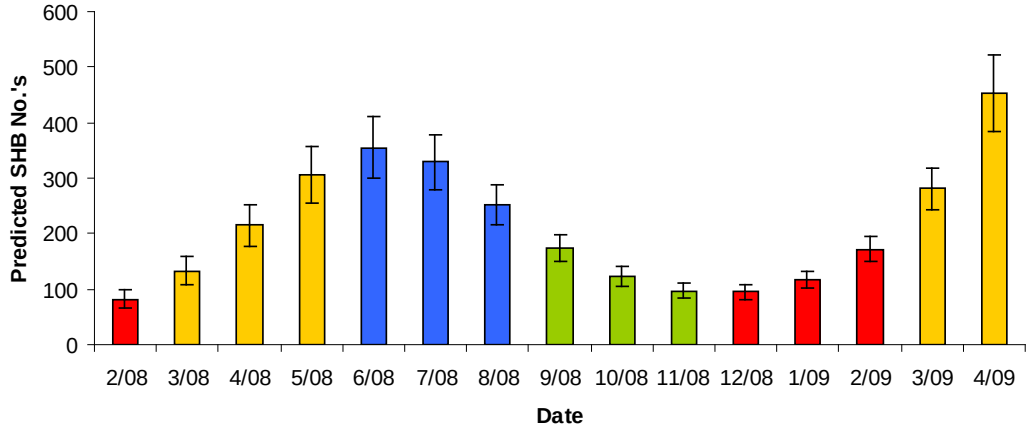

Figure 32. The predicted total mean number of SHB (inside plus outside hive) for each hive. Colours show seasons, red-summer, autumn-orange, winter-blue and spring-green and bars represent the standard errors of the means.

For the proportion of SHB found outside the hive, the sinusoidal trend over month was significant ($P < 0.01$) but there was no significant seasonal trend after adjusting for the sinusoidal trend ($P < 0.1$) nor overall linear trend with month ($p < 0.1$). There was some random variation across months about the **sinusoidal trend.**

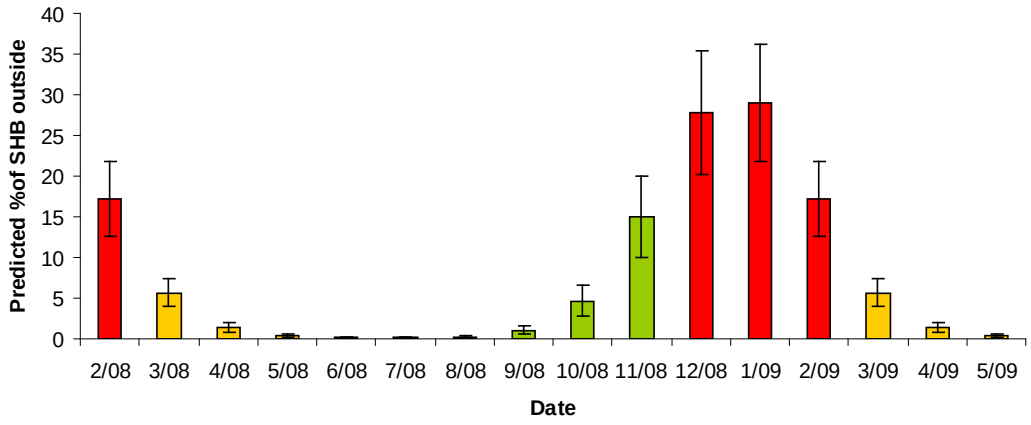

Figure 33. The predicted mean percentage of SHB found outside the hive relative to the total number of SHB for each hive. Colours show seasons, red-summer, autumn-orange, winter-blue and spring-green and bars represent the standard errors of the means.

Discussion

The sinusoidal pattern observed in the SHB population strongly reflects patterns in temperature change. However a lag period exists between the curves. At higher temperatures emergence is faster, with the life cycle from egg to emergence taking 49 days at 17 to 24ºC (Neumann *et al.*, 2001b) and 32 days at 30ºC (Schmolke, 1974). Higher temperatures also result in SHB laying more eggs, with maximum egg-laying rate occurring in the range of 25 to 35ºC (See Trial 1). Therefore at the higher temperatures recorded (See Appendix 2) the rate of increase in SHB population numbers is increased.

The lag is a result of the time taken for the SHB to develop from egg to adult. Population numbers are noted to increase in December and continue at an accelerated rate until May. Suggesting successful reproduction was starting with eggs being laid in October to initiate this pattern. Other factors may influence SHB breeding as well, including day length, the strength of the hives in the locality and availability to suitable breeding conditions such as dead hives and unclean extraction plants.

The survival of SHB also affects the sinusoidal SHB population pattern. In the same way we see a rapid increase in SHB population between December and June, a similar decrease in population numbers occurs from July to November. With declining temperatures SHB breeding and development slows. Between 15ºC and 20ºC egg laying is greatly reduced (See Trial 1) and below 15ºC SHB stop laying eggs (See Trial 1). There is an accelerated reduction in SHB replacement during this time. SHB have a maximum life expectancy of 6 months (Lundie, 1940; Ellis, 2003a). Therefore we see a natural decline in numbers as SHB reach the end of their life. Different seasonal patterns and local climatic conditions would cause some variation to this pattern.

From April through to September, the cooler months of the year, almost all the SHB were residing within the hives, with less than 1% of total SHB found outside. It is suspected that the warmth of the colony draws the SHB inside the hive and SHB have been observed in the middle of wintering bee clusters (Eischen, 1999; Pettis and Shimanuki, 2000). In late January/early February, when mean temperatures are at their yearly maximum (See Appendix 2), almost half (44%) of the SHB were outside the hive, with one hive recorded as high as 70%. From November through to February the proportion of SHB outside the hive was higher than 10%. It is suspected this would change between climatic areas with warmer regions having greater than 10% of SHB outside of the hive for a longer period of the year and cool regions a shorter period. This implies an under the hive SHB control device would be better suited to warmer/hot environments providing greater assistance where SHB are a larger problem to beekeepers.

The micro-climate at the hive entrance also appears to influence the number of SHB outside. As observed for April and May 2009 the hives with entrances with full sun exposure, resulting in warmer localised micro-environments around the entrance, had considerably more SHB outside as compared to those hives and entrances in the shade.

SHB in hives are continually being harassed by the bees in the colony, often resulting in their encapsulation by the bees (Ellis *et al.*, 2003a; Ellis *et al.*, 2003b). SHB seek refuge in small cracks (Neumann and Elzen, 2004) or under the bottom board of commercial hives (Lundie, 1940) to avoid bee harassment (Schmolke, 1974; Neumann *et al.*, 2001a) but remain within easy reach of food and warmth. Ellis et al.(2003a) noticed when trialling screen bottom boards that SHB often congregated outside under the mesh and suggested targeting SHB there with a below-hive trapping device. It appears the environment outside the hive entrance satisfies these requirements when conditions are sufficiently warm.

Interestingly the proportion of SHB found outside the hive peaked when the total SHB population was near its lowest levels for the year. With bee populations peaking through summer it is possible that the high bee to beetle ratio increases harassment levels inside the hive. Other factors possibly influencing the number of SHB outside the hive include the aggressiveness of the bees, hive health, bee genotype,

the hive situation (on grass, concrete or on toad stands), and available harbourage locations in the hive.

Implications

The data shows that during the hotter summer months a substantial proportion of the SHB population move outside the hive. The implication for beekeepers is that the use of control techniques that target SHB while outside the hive, including the use of chemical control strategies may provide another option to effectively reduce SHB numbers during the hotter months of the year. The time when honey bee colonies are at most risk from SHB. The use of chemicals outside the hive would minimise the risks regarding hive contamination and residue issues that arise from internal chemical hive treatments.

The seasonal population fluctuations provides further evidence to what was observed in trial 3 regarding the seasonal effects on the breeding cycle and population dynamics of SHB.

Recommendations

Knowing that SHB spend a substantial amount of time outside the hive provides opportunities for new control strategies, including chemical controls, to be developed and implemented. This area of research warrants investigation.

Trial 5 – Treatments

Methodology

Sixteen full depth frames were obtained from four “dead out” nucleus colonies of European honey bees that had been “slimed” by SHB larvae at the University of Western Sydney, Richmond. No active bees remained in the colonies. The slimed out colonies were transported to Bathurst on the 6 March 2009 during which the last of the SHB larvae were leaving the hives to pupate. Four frames were randomly grouped together for each treatment. There were three treatments and a negative control (i.e. 16 frames in total). A mix of wax and plastic foundation types of varying age and condition was present amongst the frames. All frames were marked with a felt pen on the top bar identifying the treatment and replicate (4 replicates per treatment). Photographs of both sides of each frame were taken prior to treatment. The treatment that required soaking commenced on the 2 May 2009 while the remaining treatments were conducted on the 3 May 2009.

Treatments tested were;

1. Frames soaked in water for 12+ h. After soaking the frames were gently rinsed with a hose to remove any loosened slime.

2. A thorough hosing of the frames with a thumb over the hose to give the frames a blast with the water to try and remove and wash out the "slime”.

3. Half a litre of bleach (125 g/L Sodium Hypochlorite) was mixed in 15 L of water. The frames were immersed for 15 min and occasionally agitated. They were then rinsed with a gentle hosing off to remove the bleach and any loosened slime.

4. Control – the frames were left undisturbed.

All excess water was shaken from the frames after treatments.

The frames were then put into four two-box hives (brood box and a super), selected for equal strength, on 3 May 2009. The four frames for each treatment were placed together into one colony by replacing the four centre frames of the honey super (no honey). Each treatment of four frames was randomly allocated to the four hives. Colony activity was low leading into winter and there was no nectar coming into the colonies.

Visual inspection was used to compare the frames post treatments. Visual inspection and photographs were used to compare the bees abilities to resurrect the treated frames.

On the 10 May 2009 and again on the 3 June 2009 all treated frames were examined and photographed.

The supers of these hives, including the treated frames, were then removed to pack the bees down for winter and placed in cold storage.

Due to some hive losses the bleach treated frames were the only ones returned to a hive in spring and were inspected and photographed on the 4 December 2009.

Results

Washing technique

Visual observations were made before and after the treatments of the slimed frames with some minor noticeable differences observed. Some parts of the comb were partially broken because of the hose pressure but the damage was of no consequence. All three washing techniques removed some of the sliming as could be seen by the discolouration of the wash water, however the majority of the slime residue remained on the frames for all treatments. The slime residue changed to a lighter colour post treatment while still wet. The slime was not easily dislodged from the combs and after treatment the majority still remained on the combs. Neither the water nor bleach solution appeared to help breakdown or dissolve the slime residue on the combs.

Bee activity

Visual observations and photographs were used to compare the differences in frame restoration by the bees. Despite approaching winter the bees entered the supers to clean out the slimed frames. At the one week post-treatment inspection (10 May 2009) more bees were observed on the slimed frames than on the other frames in the supers for all treatments and most of the slime residue had been removed. Where the combs were very old and black, or damaged by wax moth, the bees had chewed the cells right back, removing large areas. A pile of debris was at the hive entrances for all colonies with slimed frames as opposed to other colonies in the apiary where no slimed frames were present. No noticeable differences between treatments were observed, with all combs being cleaned up and chewed back where necessary.

The majority of the cleaning up of the frames occurred during their first week in the colony. Photographs taken a month after treatment (3 June 2009) showed only small amounts of further cleaning had occurred. With the onset of winter very few bees remained in the super, with bees predominantly in the brood box. No comb rebuilding (new wax) was observed on any of the slimed frames.

Honey contamination

Due to an exceptionally dry autumn, winter and spring very little food (nectar and pollen) was available for the bees and many of the colonies died. Only one of the honey supers which contained the frames that had been treated with bleach was returned to a colony post winter. Inspection of these frames seven months after treatment (4 December 2009) showed the bees rebuilt and resurrected the structure of the comb with fresh wax. Nectar was being stored in the regenerated combs with no noticeable affects from the previously spoilt honey and there was no evidence of nectar/honey fermentation occurring.

Presented below are photographs of one side of each of the 4 frames for each treatment taken at different times throughout the trial. The order of the photos is:

- Top left, taken on the 3 May 2009 except for the soaked treatment taken on the 2May 2009 (before treatment).
- Top right, taken on the 10 May 2009 (one week in hive).
- Bottom left, taken on the 3 June 2009 (one month in hive).
- Bottom right (for bleach treatment only) taken on the 4 December 2009 or various.

Treatment – Bleach wash

Figure 34. Bleach washed – frame 1.

Figure 35. Bleach washed – frame 2.

Figure 36. Bleach washed – frame 3.

Figure 37. Bleach washed – frame 4.

Treatment – Soaked in water for 12 + h

Figure 38. Soaked in water for 12 + h – frame 1. Bottom right shows debris at hive entrance (3 June 2009).

Figure 39. Soaked in water for 12 + h – frame 2. Bottom right shows frames being soaked

Figure 40. Soaked in water for 12 + h – frame 3.

Figure 41. Soaked in water for 12 + h – frame 4.

Treatment – Hosed

Figure 42 Hosed – frame 1.

Figure 43. Hosed – frame 2.

Figure 44. Hosed – frame 3.

Figure 45. Hosed – frame 4.

Treatment – Control

Figure 46. Control – frame 1.

Figure 47. Control – frame 2.

Figure 48. Control – frame 3.

Figure 49. Control – frame 4.

Discussion

Visual examination of the frames indicated that there was no difference between any of the treatments tested to clean slimed combs and the control. Some of the slimed frames had plastic foundation that appeared to withstand the treatments better and also remained more intact. If comb damage was so severe that comb recovery was not an option then plastic foundation frames could be easily cleaned by melting or scraping back the cells from the foundation before reuse. Other treatments and/or methods may be more effective but further investigation is required.

The speed at which the bees took to cleaning up the slimed frames was surprising, considering the conditions and the time of year they where placed into the colonies. One week after treatment no differences were apparent. Perhaps if additional inspections had been undertaken during the first week some differences may have been observed. Leading into the cool tablelands winter at Bathurst. minimal bee activity in the honey super, especially since they were empty of honey, could be expected. The bees did not require this space for any purpose such as nectar or pollen storage. However it appears the slimed combs caused distress to the colony, driving the bees to actively clean them up. After a week in the hives the bees had removed the majority of the slimed material on the frames. The time of year the trial was done did not seem to prevent bees from cleaning the slimed frames; in fact, it may have been advantageous in that bees did not have many other activities at this time. Heading into winter may have also minimised the chance of the colony succumbing to SHB because of low SHB activity and reproduction in the cooler months.

Ideally the recovery of hive equipment by bees should be done at times or in areas with low SHB levels. This minimises the risk of the cleaning hives succumbing to SHB because of the slimed material, stress and hive manipulations. Unfortunately it is often difficult or impractical to utilise such locations or periods but in areas with very few SHB, equipment recovery could be achieved throughout the year with minimal risk.

The bees tended to chew back the old dark cells on frames more severely. For these to be returned to being useful productive frames the bees have to rebuild much of the comb. Producing wax uses honey, resulting in lost hive production. Recovering the slimed combs would be similar to replacing the frame or renewing the foundation but with the disadvantage of the old combs harbouring diseases. The economics in resurrecting old combs is questionable and it would be more sensible to remove the old disease-harbouring combs and replace with new foundation or a whole new frame. This follows good beekeeping practise of replacing frames regularly.

Schmolke (1974) wrote of a beekeepers experience where he had an extremely heavy infestation of SHB adults in his hives. He returned some weeks later to remove honey only to find the SHB numbers had declined to more normal levels. On precautionary advice the extracted honey from these hives was kept separate. After a few weeks in storage the honey started to ferment. Unfortunately for this trial the bleach treated slimed frames never accumulated adequate honey to extract due to the poor spring and summer seasons. No assessment could be made on whether the honey would be contaminated, but no evidence of nectar/honey fermenting was observed in the combs on 4th December 09.

The trial only looked at frames with no SHB larvae present. For frames infested with larvae it is recommended that control of the larvae be achieved before placing them in a hive for cleaning. Experience has shown that a strong colony of bees is capable of removing small SHB larvae from infested frames. With larger SHB larvae the bees seem unable or unwilling to remove them, even in a strong hive. Large numbers of SHB larvae on slimed frames being placed in a colony to be recovered could, if severe enough, result in further SHB infestation and/or the hive loss. The amount of slimed material a hive can clean will depends on the strength of that hive. Caution should be given to overloading a colony with slimed material. Too much at once could be detrimental to the colony. Too

much slimed material will distract bees from other tasks in the hive therefore costing the hive in some way and could even result in SHB taking over the colony or causing the colony to abscond.

Another factor to consider is side affects of putting bees to work and creating stress in a colony approaching winter. Handling and opening hives in late autumn and winter has been shown to be associated with high levels of *Nosema* (Hornitzky, 2008). Perhaps placing slimed frames into bee colonies will promote *Nosema* infection This may have contributed to the loss of some of the hives used in the experiment. It is also possible that they died out due to the extreme seasonal conditions and lack of food. As many other colonies not exposed to slimed frames also died post winter, mainly through starvation.

Whenever beekeepers move material between hives there is always a risk of transferring diseases such as American foul brood disease (AFB) which is easily spread as spores. The risk of disease spread is heightened by the difficulty in identifying AFB symptoms in a colony infested with SHB larvae. This is because the resulting slimy mess masks symptoms of AFB that may have pre-disposed the colony to SHB in the first instance. Often stress and decline of a hive is caused by a disease enabling SHB to overrun the colony (White, 2003).

Another issue needing mention is the human health risk that the yeast *K. ohmeri* can pose. There have been a small number of reported infections and deaths overseas attributed to *K. ohmeri* in severely immuno-compromised individuals, both young and old (Yang *et al.*, 2009; Barros *et al.*, 2009; Chiu *et al.*, 2010; Han *et al.*, 2004). The yeast can cause fungaemia (infection in the blood), funguria (yeast in the urine), endocarditis and peritonitis (Yang *et al.*, 2009). The use of appropriate protective equipment, including water proof gloves and a face shield, when handling slimed bee equipment is recommended especially for operators with suppressed immunity, to minimise potential exposure to the yeast.

Implications

It was demonstrated that bees are quite capable of cleaning slimed frames for continued use in beehives with no observed contamination or hive health issues. It was also observed that no benefit was to be gained from washing frames, either in water or diluted bleach, prior to returning them to hives.

The practical implication for the beekeeper is in knowing that, although SHB can be very destructive to a honey bee colony, hard ware such as hive boxes, lids, excluders and bottom boards can be readily recovered for subsequent use. If frames are slimed but the comb integrity is still good there is no need for their destruction as they can be returned to a functioning state by the bees.

There are risks associated with the transfer of any equipment between hives. Of particular concern is the possible spread of undetected AFB, which may have induced the SHB damage by weakening the hive and this needs to be considered.

Recommendations

Beekeepers should be encouraged to inspect frames thoughtfully until they can adequately gauge for themselves what level of damage is recoverable and what is not. Economic considerations need to be taken into account at all times.

Research needs to look at alternate ways to remove the slime (fermented honey) from the combs and equipment so that spoilt equipment can be easily and safely recovered for further use.

The human health risks associated with the yeast *K. ohmeri* also need to be investigated so beekeepers can me made aware of the potential dangers, if any, and what precautions might be necessary when handling slimed equipment.

Appendices

Appendix 1. Observed maximum and minimum temperatures and humidities as recorded over the 24h surveillance periods at 30 minute intervals, from a weather station located approximately 300 m from the trial location.

Surveillance date	25/2/08	4/4/08	2/6/08	1/7/08	31/7/08	2/9/08	1/10/08	4/11/08	2/12/08	2/1/09	9/2/09	5/3/09	7/4/09	4/5/09
Max. temperature C°	27.5	24	18	20	23	22	29	22.5	32	29	23.5	28	25	25
Min. temperature C°	17.5	4.5	14.5	4.5	3	5	8.5	13	13.5	15	17.5	7.5	8.5	8.5
Max. humidty %	93	96	96	90	92	96	92	90	95	86	95	96	96	96
Min. humidty %	53	33	81	28	24	30	19	52	18	36	68	24	42	41

Appendix 2. Average monthly maximum and minimum temperature and rainfall for Richmond - UWS Hawkesbury.

Created on [21 Oct 2010 01:02:16 GMT+00:00]
067021 RICHMOND - UWS HAWKESBURY
Commenced: 1881
Last Record: 2010
Latitude: 33.62 Degrees South
Longitude: 150.75 Degrees East
Elevation: 20 m
State: NSW

Statistic Element	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual	No.of Years	Start Year	End Year
Mean max. temp. C°	29.4	28.9	27.1	23.8	20.3	17.4	17.3	18.9	22.1	25	27.1	29.1	23.9	60	1907	1975
Mean min. temp. C°	16.8	16.8	15	11.3	7.3	4.7	3.2	4.4	7.1	10.5	13.1	15.5	10.5	60	1907	1975
Mean rainfall (mm)	95.2	95.4	87.3	66.8	58.5	60.8	45.8	43.1	42.7	57.6	72.3	75.2	799.9	129	1881	2010

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Small Hive Beetle Biology

— *Producing control options* —

by Nicholas Annand

Publication No. 11/044

The Small Hive Beetle (SHB) was first identified in Australia in 2002. Since then it has become a major pest of honeybee hives.

Many control strategies are already in use in Australia to minimise the impact of SHB and include modifications to hive designs, improved beekeeping techniques and hygiene procedures. However SHB continues to cause large-scale economic losses within the industry. It is now clear that a better understanding of the biology of the SHB is necessary if beekeepers are to effectively manage this pest.

This project highlights the biological and behavioural characteristics of SHB that can be directly related to hive health and management. The knowledge can be used to enhance the effectiveness of current control strategies and to provide the basis for new and improved control strategies for the commercial and amateur beekeeping industry.

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Cover photo: Small hive beetle larvae in a hive

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