NSW GOVERNMENT | Department of Primary Industries

MANAGING AFB

Guidelines for the identification and management of American foulbrood – a fatal disease of honey bee colonies.

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www.dpi.nsw.gov.au

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INTRODUCTION

This field guide has been designed to assist beekeepers in the identification and management of American foulbrood (AFB) which is a disease caused by the bacterium *Paenibacillus larvae*.

Honey bees are susceptible to a range of pests and diseases, most of which are not lethal to the colony but can cause substantial loss in production. One disease, AFB, is lethal to bee colonies and modern beekeeping practices have increased its prevalence.

While some assistance is available to beekeepers, the field diagnosis of the disease is largely up to the beekeeper. Unlike other livestock, beekeepers do not have access to experts such as veterinarians to assist with field identification. Thus the task of learning about AFB, being able to identify the various stages of AFB and know how to manage AFB is primarily the beekeeper's responsibility.

The population of a honey bee colony varies due to the capacity of the queen to lay eggs, the nutritional resources available (nectar and pollen), plus the disease status of the colony. A full inspection of all brood combs in all colonies in the spring and autumn is considered good practice. Underperforming colonies at any other time of the year should be inspected. This enables the beekeeper to identify which colonies require re-queening,

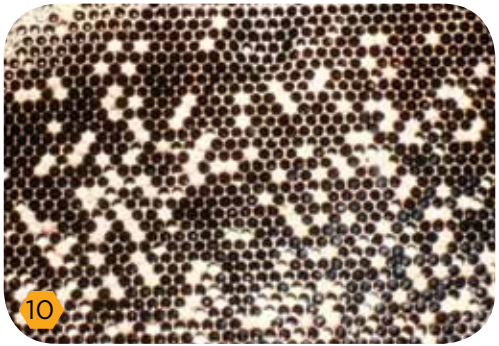
what diseases or pests are present, and provides information on the general condition of the colony (stored honey, age of combs).

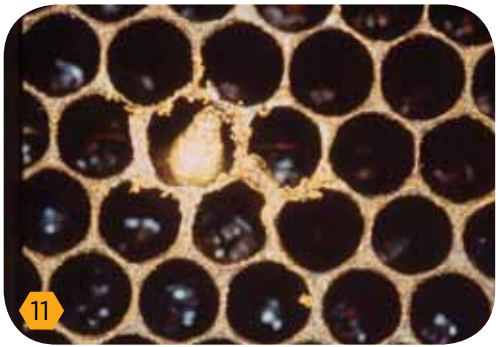
Hive inspection

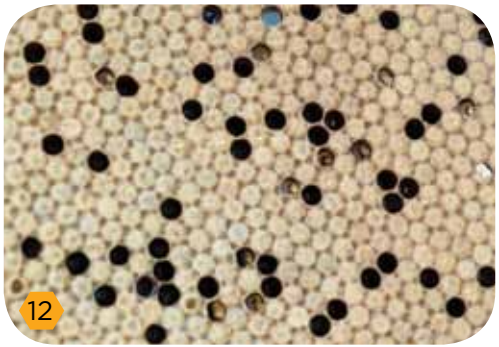
1. opening a hive
2. removing a comb
3. removing bees
4. inspecting brood cells

5. healthy larvae
6. healthy capped brood
7. capped honey
8. pollen in cells (bee bread)

Other conditions and diseases affecting honey bee brood may be confused with the diagnosis of AFB. These diseases may include European foulbrood, sacbrood, chalkbrood and chilled brood.







9. EFB – early stages
10. EFB – advanced stages
11. sacbrood
12. chalkbrood

Antibiotic use in bee hives

Antibiotics are not recommended or lawful to use in NSW to control AFB. One of the options to control European foulbrood (EFB) is to treat the colony with an antibiotic (Oxytetracycline) to break the disease cycle. In most cases this enables the bees to clean up the disease and improve in health. Both AFB and EFB are bacterial diseases, but that is where the similarity stops. Some international information sources suggest the use of antibiotics to manage AFB. The reasons for not adopting this approach are stated in Table 1.

Table 1. Fundamental differences between EFB and AFB

EFB	AFB
Cause: *Melissococcus pluton*	Cause: *Panenibacillus larvae*
Occurs seasonally, associated with nutritionally stressed colonies and the cooler climatic conditions in early spring.	Honey bee colonies are susceptible to AFB under all conditions and seasons.
The bacteria are always present.	Possible to eliminate the causative bacteria from the system.
Highly susceptible to antibiotics and does not form a spore.	The spore stage of the disease is not affected by antibiotics.

EFB	AFB
Only one treatment of antibiotics combined with good breeding conditions (nectar and pollen) is necessary to control the disease.	60% of the colonies treated will show signs of the disease again within 1 to 14 months, irrelevant of breeding conditions. Necessity to treat infected colonies multiple times during the year.
Antibiotic residue risk with the first honey extracted after treatment, thereafter the residue risk is minimal.	Substantial risk of increased antibiotic residue in all extracted honey.
In many years the presence of EFB is not serious enough to warrant the use of antibiotic treatments.	Once a program of using antibiotics to suppress AFB has been started, a high % of colonies would present with AFB symptoms if treatments were stopped.
Legal to use antibiotics to treat a colony infected with EFB.	Illegal to use antibiotics to control AFB.

ABOUT THE DISEASE

The essential facts about AFB

- Caused by the bacterium *Paenibacillus larvae*.
- It is not a stress related disease and can infect the strongest to the weakest colony in an apiary.
- It is not highly contagious and is primarily spread by beekeepers exchanging equipment between infected and healthy hives or by bees robbing infected weak/dead colonies.
- It is a spore forming bacteria with the spore being able to survive for decades.
- The spore stage is extremely resilient to heat, cold and drying.
- The spores can survive on **all** stored bee hive components, including honey.
- Only 6 to 10 spores are required to cause an infection in a day old larvae.
- A bee larvae less than 24 hours old is the most susceptible stage to be infected by AFB. The younger the larvae, the more susceptible and less spores required to cause infection.
- The bacteria rapidly multiply after the larvae have been capped.

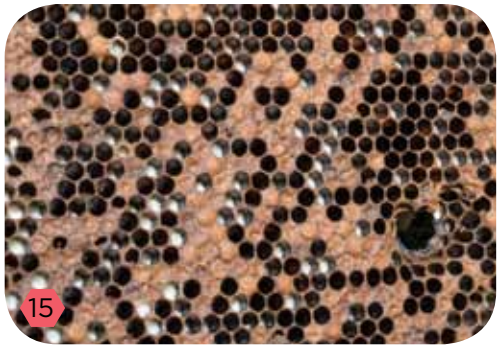
- 2.6 billion AFB spores are produced in one infected larvae.
- The disease is fatal to the bee colony.

13.stored equipment
14.day old larvae

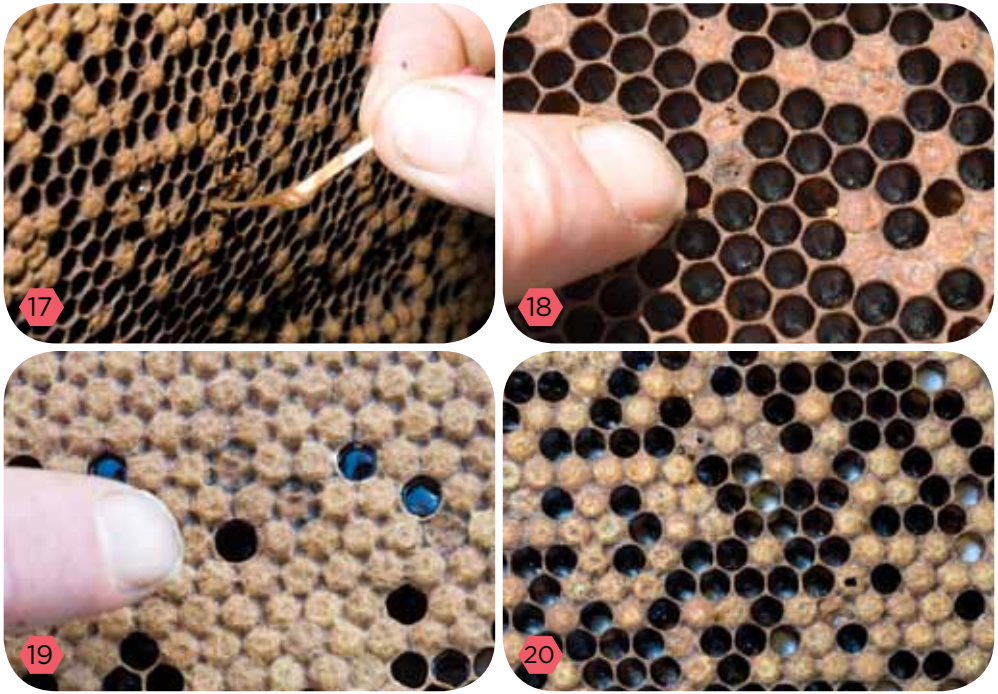
SIGNS AND SYMPTOMS

Initial Infection:

- small number of infected brood (majority of brood healthy)
- odd brood cell with soft brown decayed brood amongst healthy brood
- a stick inserted into decayed brood ropes out
- cappings discoloured, darker than cappings on healthy brood
- cappings sunken
- cappings partly chewed or perforated (an attempt by nurse bees to remove cappings)



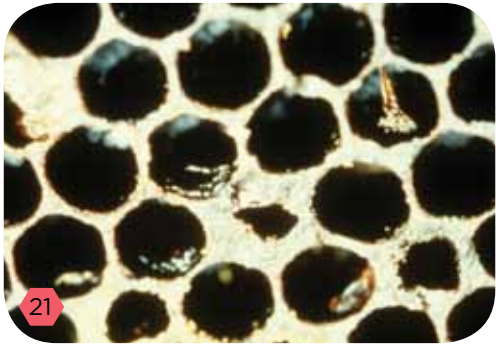
15. scattered diseased brood amongst healthy cells
16. early decayed brood



17. roping infected brood
18. dark sunken cappings
19. dark sunken cappings
20. cappings partially chewed

Intermediate Infection

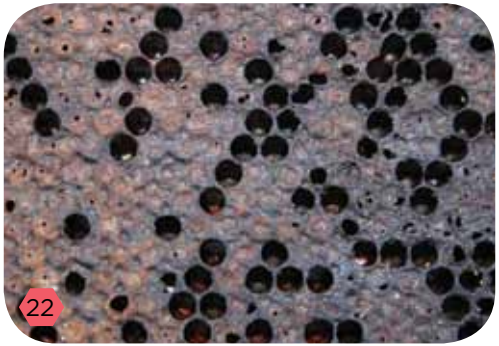
- adult bee population still reasonable
- numerous sunken dark cell cappings obvious amongst healthy brood
- infected brood continues to rope
- increasing number of scales



21. brood cells with scale

Advanced Infection

- adult population declining
- over 50% of brood infecteds
- dark sunken cell caps common
- infected brood drying out
- scale common
- distinct odour
- colony dies



22. large area of sunken, dark cappings
23. large numbers of scale

COLONY SUSCEPTIBILITY

With many of the pests and diseases of honey bees, various conditions need to prevail for the problem to occur. There are no environmental or nutritional predisposing conditions necessary for the development of AFB. Both strong and weak colonies are susceptible to AFB.

If a bee larvae less than 24 hours old is fed 6 to 10 AFB spores in its food by nurse bees this then may cause the larvae to be infected and die from AFB. The bacteria will multiply, producing approximately 2.6 billion spores in each dead individual. The action of the house bees in cleaning up the diseased brood will lead to more young larvae being infected.

The number of AFB spores and population of bees in the colony will influence the rate of spread of the disease within the colony. The decline in the colony population may happen slowly over several months or very quickly, due in part to the number of spores in the hive. The higher the number of spores in the initial infection, the quicker the colonies death.

Some colonies show some resistance to AFB. This is usually referred to as hygienic behaviour which is the capacity of adult bees to detect diseased brood, uncap cells and remove diseased brood.

SAMPLES FOR DIAGNOSIS

It is very important to confirm AFB. Signs and symptoms of AFB can easily be confused with EFB, sacbrood, chalkbrood, over-heated or chilled brood. Accurate diagnosis of the presence of AFB can be obtained by submitting samples of suspect diseased material to the State Veterinary Diagnostic Laboratory.

Two types of samples are acceptable for the diagnosis of AFB; smears of diseased brood on microscopic slides (1) and sections of brood comb containing diseased brood (2). Smears are preferred for AFB diagnosis as they are non-destructive as compared to comb samples, they are easily sent via the post and they are quickly processed by the laboratory.

Honey samples (3) provide an effective means of tracing infection sources when AFB spores are detected in honey. However, AFB positive honey is insufficient to make a diagnosis of AFB. A diagnosis of AFB can only be made based on the detection of diseased brood infected with AFB spores.

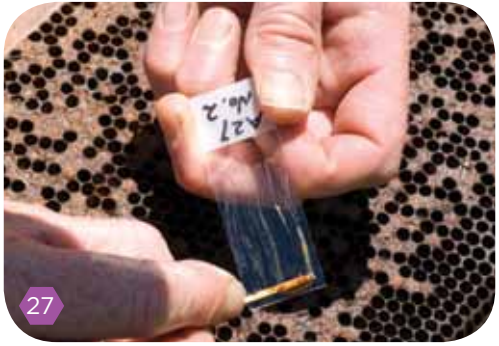
1. Microscope slide sample

- label the slide with registration number and hive number
- remove 3 or 4 infected larvae and place on slide
- pulp the larvae and wipe off excess from slide
- allow to dry
- package to send to lab
- include name, address and any other details you regard as relevant.

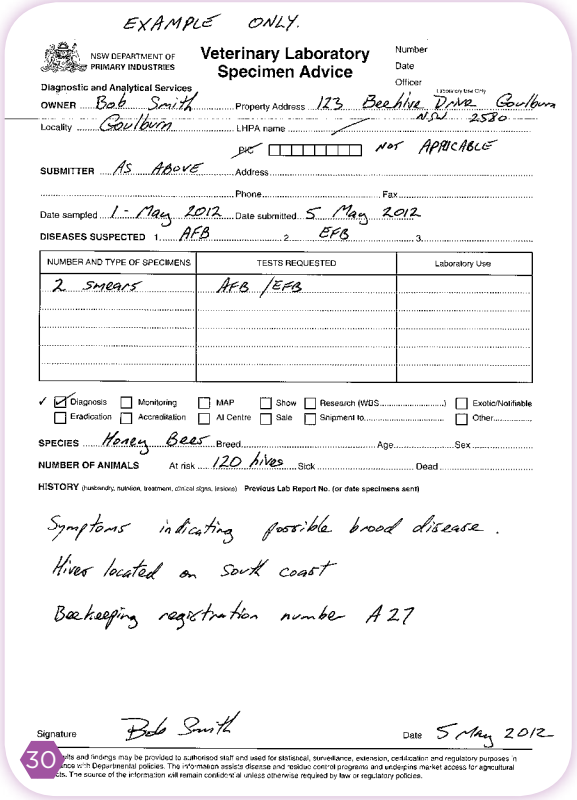
24


25

24. label slide
25. 3 to 4 infected larvae



26. pulp larvae
27. remove excess larval remains
28. allow to dry
29. pack slide



EXAMPLE ONLY.

NSW DEPARTMENT OF PRIMARY INDUSTRIES

Veterinary Laboratory Specimen Advice

Number
Date
Officer

Diagnostic and Analytical Services

OWNER Bob Smith Property Address 123 Beehive Drive (Unless by-law City) Goulburn

Locality Goulburn LHPA name NSW 2580

PIC ☐☐☐☐☐☐☐☐ NOT APPLICABLE

SUBMITTER AS ABOVE Address

Phone Fax

Date sampled 1 - May 2012 Date submitted 5 May 2012

DISEASES SUSPECTED 1. AFB 2. EFB 3.

NUMBER AND TYPE OF SPECIMENS	TESTS REQUESTED	Laboratory Use
2 smears	AFB / EFB	

✓ ☑ Diagnosis ☐ Monitoring ☐ MAP ☐ Show ☐ Research (WDS.....) ☐ Exotic/Notifiable
☐ Eradication ☐ Accreditation ☐ AI Centre ☐ Sale ☐ Shipment to..... ☐ Other.....

SPECIES Honey Bees Breed Age Sex

NUMBER OF ANIMALS At risk 120 hives Sick Dead

HISTORY (husbandry, nutrition, treatment, clinical signs, lesions) Previous Lab Report No. (or date specimens sent)

Symptoms indicating possible brood disease.

Hives located on South coast

Beekeeping registration number A27

Signature Bob Smith Date 5 May 2012

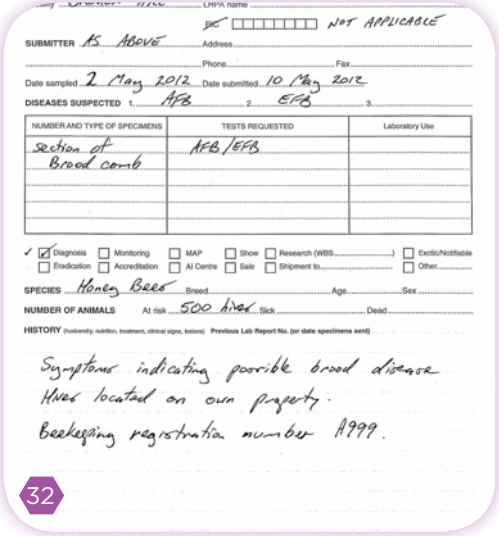
30 Results and findings may be provided to authorised staff and used for statistical, surveillance, extension, certification and regulatory purposes in accordance with Departmental policies. The information assists disease and residue control programs and underpins market access for agricultural products. The source of the information will remain confidential unless otherwise required by law or regulatory policies.

30. fill out lab submission form

2. Comb sample

- cut a piece of comb containing infected larvae (about 8 × 8 cm)
- wrap in paper (not plastic) and package in cardboard to avoid crushing of sample
- include your name, address and any other details you regard as relevant.


31


NOT APPLICABLE

SUBMITTER AS ABOVE Address

Phone Fax

Date sampled 2 May 2012 Date submitted 10 May 2012

DISEASES SUSPECTED 1. AFB 2. EFB 3.

NUMBER AND TYPE OF SPECIMENS	TESTS REQUESTED	Laboratory Use
section of Brood comb	AFB/EFB	

✓ ☑ Diagnosis ☐ Monitoring ☐ MAP ☐ Show ☐ Research (WBS) ☐ Exotic/Notifiable
☐ Eradication ☐ Accreditation ☐ AI Centre ☐ Sale ☐ Shipment to ☐ Other

SPECIES Honey Bees Breed Age Sex

NUMBER OF ANIMALS At risk 500 hives Sick Dead

HISTORY (husbandry, nutrition, treatment, clinical signs, lesions) Previous Lab Report No. (or date specimens sent)

Symptoms indicating possible brood disease
Hives located on own property.
Beekeeping registration number A999.

32

31. cut out piece of comb with infected brood package
32. fill out lab submission form

3. Honey sample

- 100 grams of honey in a secure jar
- pack to send to the lab
- include name, address and any other details you regard as relevant

Honey samples are tested for AFB spores. The preferred honey sample is collected following an extraction of multiple hives, although samples collected from individual hives may also be tested. A positive result is usually expressed as a +1, +2 or +3.

Spore count	% chance of having visual symptoms of AFB
+1	56
+2	80
+3	100

Honey samples are used to provide general information on the disease (AFB) status of a group of bee hives. This sampling technique does not replace a thorough brood inspection in spring and autumn or the inspection of poorly performing hives. A negative result provides a good indication that AFB spores are not present in the hives sampled but does not necessarily mean that there are no AFB spores in the hives sampled.

33. honey sample
34. petri dish with AFB culture

ADDRESS OF LAB FOR SAMPLES

State Veterinary Diagnostic Laboratory

Officer-in-charge

Courier: Woodbridge Road, Menangle NSW 2568

Post: Private Mail Bag 4008, Narellan NSW 2567

MANAGING THE SPREAD OF THE DISEASE

There are several means by which AFB is spread. They include interchanging of contaminated equipment, feeding honey and pollen containing AFB spores, contaminated apiary equipment and bees robbing honey from weak or dead contaminated colonies (either managed or feral). The following information is provided to put in perspective the relative risk associated with each factor that have traditionally been associated with the spread of AFB. WARNING, a negligible risk rating does not mean that it is impossible to obtain an infection by this activity but rather the risk is very low.

Risk rating (negligible, low, moderate, high, extreme)

1. Contaminated combs EXTREME
2. Contaminated bee boxes, lids, bottoms MODERATE
3. Feeding contaminated honey and/or pollen HIGH
4. Robbing diseased hives (managed or feral) HIGH
5. Collecting swarms LOW
6. Buying queen bees NEGLIGIBLE
7. Bees wax NEGLIGIBLE

1. Contaminated combs

The transfer of combs contaminated by AFB spores will most likely cause disease in the colony into which the combs have been transferred. The AFB risk is extremely high and most likely will cause an infection. This is one of the primary reasons why a barrier system should be considered so as to restrict the cross contamination of AFB spores between apiaries.

2. Contaminated bee boxes, lids and bottoms

The risk rating of moderate for the re-use of hive components depends on the amount of AFB contaminated wax and honey on the material.

Removal of wax and propolis by washing and scraping will reduce the spore contamination load. Repainting the inner surfaces will also reduce the available spores to a colony. These practices will not sterilise the equipment. Gamma irradiation will sterilise all equipment.

3. Feeding contaminated honey and pollen

Honey and pollen harvested from hives with AFB will contain AFB spores. If this diseased food is fed to one day old larvae it will cause an infection. It is not a good practice to feed honey to bees and it is strongly recommended to gamma irradiate all pollen prior to feeding to bees.

4. Robbing diseased hives either managed or feral

The rating of high indicates that this is a major pathway of hives becoming infected with AFB. Even so a set of circumstances need to prevail for this to be a significant risk factor.

- Your bees are in a robbing mode, i.e. at the end of a nectar flow, or little or no nectar available in the field.
- Another colony within flying distance has succumbed to an AFB infection and can no longer defend itself. Flying distances will vary according to air temperature and wind.
- Diseased honey is robbed from the infected colony and returned to your colony. If the infected honey is then fed to day old larvae than an infection in your colony may result. If the infected honey is fed to adult bees only, and not larvae, or the infected honey is stored and does not come into contact with day old larvae, it is possible for the robbing colony to escape or delay an infection. As an example field bees from 10 hives out of 100 in your apiary find a recently deceased hive heavily contaminated with AFB. These 10 colonies may or may not contract AFB as a result of contaminated honey being returned to their colony. This will depend on the amount of contaminated honey robbed and concentration of spores within that honey, plus whether it is fed to a day old larvae on return. The chances of these 10 colonies contracting an AFB infection will proportionally increase with the amount of diseased honey collected and number of spores consumed.

5. Collecting swarms

Swarms are a means by which a colony reproduces itself. Swarming of a colony occurs primarily in spring after colonies expand their population after winter. Medium to heavily infected colonies with disease are not likely to reach this condition. A colony with a light AFB infection may swarm. In this case, by definition, the spore load will be lower than medium or heavily infected colonies.

Spores could be transported by the swarm in contaminated honey. Once a swarm finds a new location to reside, the bees will consume this 'stored' honey to produce wax. In this case, by the time day old larvae are present, the chances of AFB spores being present in the colony are extremely low.

35. swarm

Swarms should preferably be placed onto foundation and/or 'dry' combs (no honey). It will be several days before there is a susceptible day old larvae in the hive. The chances of AFB spores being fed to the day old larvae in this case are extremely low.

Thus, collecting swarms possess an extremely low risk in perpetuating AFB infections.

6 & 7. Queen bees and foundation

There have been no reported cases where AFB infections can be traced to the introduction of queens in mailing cages or the use of beeswax foundation made from contaminated wax. Queen candy used to feed queens and escort worker bees in the mailing cages should be made with gamma irradiated (sterilized) honey.

36. queen bee in mailing cage

Barrier systems

One of the principal means by which AFB is spread is via contaminated equipment. Unfortunately it is not always possible to know if equipment is contaminated with AFB spores. One system of management worth implementing is a 'barrier system'. The working definition is simple – 'there is some degree of segregation of hives or apiaries within a beekeeping operation whereby material from one hive/group/apiary is only interchanged with that hive/group/apiary'.

The most popular barrier system is where each apiary is identified and managed as a separate entity where boxes, frames etc. are only interchanged within each apiary and not between other apiaries. Thus, in the event of finding AFB in one hive, you can be reasonably confident that the disease has not been transferred to another apiary by the transfer of contaminated equipment.

If you are buying second hand equipment then it is always a good practice to keep this material separate (barrier system approach) from all the other beekeeping equipment (boxes, combs, lids, bottoms) for at least twelve months. This should include at least two extractions of honey from any supers of combs.

Barrier systems are a very common management strategy in agricultural systems, particularly in intensive livestock businesses. Commercial beekeeping is an intensive livestock system, with the opportunities for pests and diseases to become a major problem. By adopting a barrier system, you will reduce the risk of spreading pests and diseases throughout the beekeeping operation via the transfer of equipment thus making the clean-up, particularly for AFB, that much more manageable.

Inspection rate/frequency

37. inspecting combs (no bees on frames)

All combs in the brood box should be thoroughly inspected for AFB at least twice a year. This is usually completed in early spring and mid autumn, although this will vary according to seasonal variations in climate and food availability. It is not recommended that hives are opened for inspection when there is no nectar available in the field, as this may promote robbing activity. When inspecting combs it is extremely important that all bees are removed from the comb so you have a clear view of all the brood cells.

Record keeping

Very few beekeepers individually number each hive, thus it is important that a permanent marker be carried whenever inspecting hives. Hives that need follow up attention or samples taken for laboratory diagnosis can then be marked on the lid, to be identified in future.

Any movement of apiaries should be recorded so a trace back is possible if AFB does occur in your hives. This may assist in the location of the original infection and identify what hives are at risk of carrying AFB spores which could cause a hive to break-down with AFB in the following months.

Managing infected hives

First assess the risk of the diseased hives infecting other hives. A weak or dead colony is unable to defend itself and is an immediate threat to healthy colonies in the area. If the diseased colonies are strong in bee numbers the urgency to process the contaminated equipment is not as great.

The second consideration is to decide what to do with the contaminated equipment. Do you burn all or some of the boxes, frames, etc, or prepare the equipment for gamma irradiation (sterilisation). Hot wax dipping boxes may also be a consideration. Each process has its advantages and disadvantages. Refer to the information on each procedure to see what options suit your situation.

The next step is to contain the disease. Diseased hives could be moved to a separate location away from healthy colonies before killing the diseased colonies and processing the material, or the diseased colonies could be killed on site.

Killing diseased hives

1. Wait until there is no more flight by field bees.
2. Seal the entrance with a hive closer, tape or a shovel of dirt.
3. Seal any holes or cracks where bees could escape.
4. Lift the lid and pour a cup of petrol over the bees/frames and close the lid. This is a quick operation. WARNING: the smoker should be kept well away from the petrol.
5. The colony usually dies within minutes. If after ten minutes the colony has not died, pour another cup of petrol in the hive.
6. The colony is now dead and the hive material can be processed.
7. An alternative method of killing bees is to mix a tub of soapy water. Shake each frame of bees into the soapy water. It is important to protect the combs that have had bees removed from robbing bees.
8. A combination of points 1 to 7 may be followed, e.g. all the combs to be kept for irradiation (sterilisation) are removed (step 7) for honey extraction or simply removed without killing the bees. Combs with bees that are to be burnt are left in the hive and the bees killed using petrol (steps 1 to 5).

Processing infected material

WARNING: It is extremely important that robbing bees do not gain access to the diseased equipment. If the AFB contaminated equipment is to be burnt, proceed to the next section. If the combs from a diseased hive are to be extracted, then a great deal of care should be maintained. If extracting combs from healthy colonies, do these first. Extract combs from AFB diseased colonies last.

Wash the extracting plant down thoroughly with water after removing the honey. Ensure this honey is not fed back to bees. When selling onto a honey packer, inform them of the origin of the honey (disease status of the hives).

PROCEDURE FOR BURNING

1. Obtain any fire permits from the local fire captain or station. **Do not** burn material on a total fire ban day.
2. Dig a hole at least 30 cm deep. (Stops wax and honey from escaping).
3. Clear combustible material away from around the pit.
4. Place the diseased material into the pit to burn.
5. Once incinerated (burnt), cover with soil.

38. burning hives in a pit

PROCEDURE FOR GAMMA IRRADIATION

Gamma irradiation has been proven to be 100% effective in sterilizing AFB infected bee hive material. A dose rate of 10 kilograys is required. The irradiated combs and boxes are readily accepted by healthy bees. The procedure for the preparation of bee hive material is as follows:

39. hive material prepared for irradiation

1. Extract all honey
2. Depopulate the hive
3. All hive material should be free of insects/spiders
4. Securely tape/strap or emlock material
5. Each lot of material not to weigh more than 25 kg
6. Place material in plastic bags or shrink wrap
7. Clearly label all parcels with your name
8. Contact irradiation plant to arrange delivery and pick up

PROCEDURE FOR WAX DIPPING

This choice of treating AFB contaminated material is only appropriate for those beekeepers with access to a hot wax dipping vat. Hot wax dipping is used to preserve timber bee boxes from dry rot.

40. hot wax vat

The procedure of using wax dipping is as follows:

1. All the bees, brood, honey and combs are removed and burnt.
2. Store the boxes, lids and bottom boards in a bee-proof area.
3. Heat wax to 160°C.
4. The contaminated equipment is immersed in the wax for 10 minutes (no less).
5. Suitable safety equipment should be worn, including heavy duty industrial rubber gloves, a full face mask, work boots, long sleeved shirt and long trousers.

ROLE OF GOVERNMENT

Legal responsibilities (Apiaries Act 1985)

- beekeepers are required to report every new case of AFB to an inspector within 24 hours.
- comply with all written directions issued by an inspector.
- do not treat a hive with antibiotics if it has an AFB infection.

Diagnostic service

- a free service to all NSW registered beekeepers. Samples of either brood comb or microscopic slides are examined for AFB spores.

Extension/Education

- provision of information sheets on various management/procedural issues on controlling AFB.
- courses on pests and diseases of honey bees.
- provide advice to individual beekeepers when requested.

FURTHER READING

NSW DPI web site:
www.dpi.nsw.gov.au

Information sheets:

Samples for bee disease diagnosis *Primefact 895*

American foulbrood *Primefact 209*

American foulbrood disease – inspection management *Primefact 39*

American foulbrood in NSW *Primefact 878*

American foulbrood – barrier systems *Primefact 824*

American foulbrood positive diagnosis – what should you do? *Primefact 744*

American foulbrood disease – sending beehive material for irradiation *Primefact 194*

American foulbrood – tracing the source *Primefact 759*

European foulbrood and its control *Primefact 1000*

Videos:

www.dpi.nsw.gov.au/agriculture/livestock/honey-bees/pests-diseases/videos

Video 1 – Examining bee hives for disease

Video 2 – Identifying American Foulbrood

Video 3 – Making an American Foulbrood disease slide

Video 4 – Destroying bee colonies with soapy water

Video 5 – Destroying hives with petrol and burning

Video 6 – Irradiating hives

Video 7 – Management strategy for American Foulbrood

Book:

Elimination of American Foulbrood Disease without the use of Drugs. A Practical Manual for Beekeepers, by Mark Goodwin. Revised Edition 2006. National Beekeepers' Association of New Zealand, (Inc.)

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