Fact sheet

Beekeeper sampling – Varroa mites

All beekeepers have a responsibility to inspect for Varroa mites. This fact sheet outlines how you can sample for Varroa mites.

Sampling for Varroa

There are three main methods used for sampling for Varroa, including:

- **external dislodgement** - samples a small percentage of the adult bee population in the hive at a particular point in time, and involves one of two release agents (alcohol – e.g. ethanol, or a very fine powder – e.g. icing sugar). Ethanol combined with vigorous shaking is the most effective of these two sampling methods.
- **acaracide-based treatments (with sticky mats)** - samples the entire hive (brood and adult bees) over a period of time. This method is generally the most sensitive sampling method, although potential issues with resistance, availability, and label restrictions mean that acaracide treatments at this stage are not available for general beekeeper use.
- **visual inspection** - samples a very small percentage of the entire hive (potentially brood and adults), at a particular point of time, and involves observing mites on bees, brood and mite-related symptoms, including Parasitic Mite Syndrome (PMS) and mite faecal droppings on cell walls), or associated damage to bees, including Deformed Wing Virus (DWV). However, this is the least sensitive method, particularly where only very low numbers of mites may be present.

1. Sampling methodology

PIRSA recommends beekeepers sample for Varroa mites:

- using the alcohol wash method (as detailed in Table 2), comprising of an alcohol wash of: (i) approximately 300 adult bees (Steps 1-3), and additional optional steps including an alcohol wash of (ii) dislodged material from 2 frames (Step 4), and an alcohol wash of (iii) approximately 100 drone brood (if present - Step 5)
- at the following frequencies and percentage of hives per apiary (as detailed in Table 1).

2. Sampling equipment

Table 2 identifies the equipment needed, which includes:

- a container (with a diameter of a least 100 mm and a height of at least 100 mm, to ensure mites are not trapped within the mass of the bees per sample). Suitable containers can be purchased or made, including:
  - the Varroa EasyCheck kit, available from suppliers including adelaidebeekeeping.com.au and nuplasapiaristsupplies.com.au
  - instructions for making a double container alcohol washing kit, at beeaware.org.au/wp-content/uploads/2014/03/Alcohol-washing.pdf
- the use of alcohol, specifically ethanol (e.g. methylated spirits) or isopropyl alcohol (e.g. rubbing alcohol).

Contact person: Michael Stedman
Email: michael.stedman@sa.gov.au
Phone: 0408 812 698
pir.sa.gov.au

Government of South Australia
Department of Primary Industries and Regions

3. Sampling tips

Sampling tips include:

- **Sampling is important** - but not to the extent of killing brood, initiating robbing or killing the queen.
- **minimise alcohol flammability** - including by: (i) diluting with water as necessary to below 70% alcohol and (ii) keeping the smoker and lighters away from alcohol.
- **maximise sensitivity** - including by only sampling nurse bees taken from the brood nest, and by sampling no more than 300 bees per sample.
- **prevent queen losses** - including by: (i) ensuring the queen is not included in the sample (as the alcohol kills the bees), and (ii) keeping the alcohol away from open hives and frames (to prevent spilling alcohol into hive).
- **practice good biosecurity**- including by: (i) collecting and disposing of dead bees appropriately, (ii) identifying samples to hives and recording details, and (iii) decontaminating after sampling each hive where mites detected/ suspected, and additionally prior to departure from each apiary.

4. Testing your bees and submitting your samples*

Once you've sampled for Varroa mite:

1. Fill out the 'Notification of suspect varroa mite detection' form (www.pir.sa.gov.au/varroa).
2. Email the form to PIRSA.beebiosecurity@sa.gov.au
3. Send your samples and form to the PIRSA Apiary Unit, 33 Flemington St, Glenside SA 5065.

*Whether or not you detect debris or mites on your sample, send your samples to PIRSA Apiary Unit.

Table 1. Recommended alcohol wash sampling frequency

Situation	Inspection timing	Recommended % hives to sample in apiary	Follow-up sampling
Where a hive that has: • progressively and/ or inexplicably weakened. • detected or suspected mites, mite-related symptoms or associated damage.	As soon as possible or as directed.	100% of: • affected/ suspected hives. • adjacent hives.	Preferably before any movement/ uniting, but typically there is limited value in repeat sampling within 15-30 days of a previous sampling.
For each hive containing bees, hive components and/ or apiary products introduced from NSW since 1 January 2022.	As soon as possible or as directed.	100% of all such hives.	
Where made aware of potential risk of being affected with Varroa.	As soon as possible or as directed.	At least 50% of each at-risk apiary.	As above, but with an option of sampling the other 10-50% of the apiary approximately 30 days after previous sampling.
Where hives used for a high-density pollination event (eg. almond pollination).	Before the event.	10% (minimum) to 50% (recommended) of each apiary.	
Beekeeper initiated mite sampling.	As soon as possible	10% (minimum) to 50% (recommended) of each apiary.	

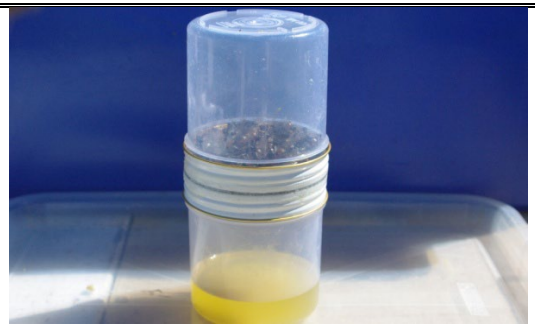
Table 2. Recommended alcohol wash sampling method

Assemble equipment:

- Instruction sheet.
- Alcohol wash unit - carry container, band, mesh lid, container ***A*** (containing sufficient of solution ***A*** per hive sample - to cover all bees; eg. approx. 150 mL/ 25 mm of solution for a 100 mm diameter container), and container ***B***.
- Solution ***A*** - 96% ethanol (eg. methylated spirits) diluted at rate 20 ml water/80ml methylated spirits.
- Filter paper (eg. 19.5 x 17 cm baby wipe).
- Bulldog clips and waterproof texta.
- Pipettes/paintbrush/cappings scratcher.
- Sealable plastic bag (eg. 17 x 15 cm bag).
- Spray mister (with approx. 100 mL solution ***A***).
- Sampling tray (eg. 41 x 24 cm plastic tray).

1. Collect sample of worker adults:

a. sample hive during brood inspections if possible to minimise decontamination requirements should mites be detected/suspected.
b. add 150 mL (25 mm) of solution ***A*** to container ***A***, and 100 mL to ½ L spray mister.
c. where possible select two brood frames that contain large areas of open or open and capped brood, and approx. 100 mature drone cells.
d. check queen is not present and shake adult bees from frames into sampling tray (put frames back if also sampling brood cells).
e. re-check queen is not on tray and tip approx. 300 bees (35 mm deep for a 100 mm diameter container) into alcohol wash container ***A***.



2. Separate mites from bees:

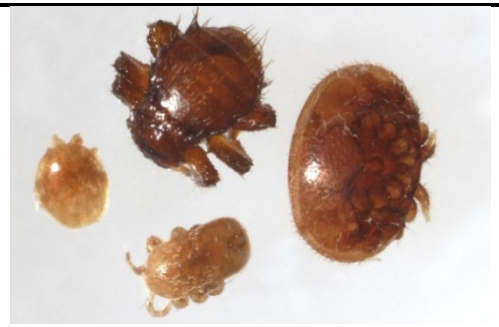
a. screw lid tightly onto container ***A***, and screw container ***B*** tightly on top.
b. invert containers so container ***A*** containing bees/brood is on top.
c. shake **up and down** vigorously for at least 60 seconds (adults), or 5-10 seconds (drone brood/debris) without pulping brood).
d. swirl from side to side vigorously for at least 10 seconds whilst repeatedly tapping container to ensure any mites trapped amongst bees/broods fall into container ***B*** on bottom.

2. Separate mites from bees (continued):

e. unscrew lid and container ***A*** with bees/brood, from container ***B*** (which is still on bottom).
f. unscrew lid containing bees/brood from container ***A***, invert container ***A*** so it's upright, and dispose of bees/brood on lid.
g. squirt 5-10 mL of solution ***A*** from mister into up-righted container ***A***, use pipette/paintbrush to recover all residual debris and place in container ***B***, and/or swirl solution to ensure all debris are entrained; then pour solution into container ***B***.

3. Filter mites and debris:

a. select new filter paper and new sealable plastic bag.
b. form filter paper into deep cone over container ***A***, ensuring filter paper overhangs outside of rim.
c. place lid firmly over filter paper on container ***A***.
d. swirl solution to ensure all debris are entrained and slowly pour solution and debris from container ***B*** through filter paper into container ***A***.



3. Filter mites and debris (continued):

e. squirt 5-10 mL of solution ***A*** from mister into container ***B***, use pipette/paintbrush to recover all debris and place onto filter paper, and/or swirl solution to ensure all debris is entrained; then pour solution onto filter paper.
f. allow filter paper to drain.
g. inspect filter paper for integrity. If not damaged, and if required for additional same hive sampling (eg. brood cells/drone brood), temporarily place filter paper over ring, bulldog clip in place, and place into the new sealable plastic bag ***A*** until reused (**Fig. 1**).

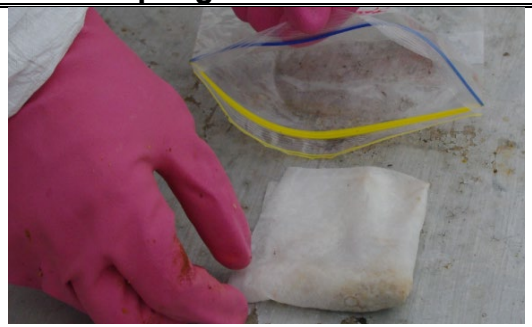
4. Collect sample from brood cells:

a. select frames as per **1. a.-e.**
b. dislodge any mites from open cells by holding frame face down over sampling tray and bumping frame against tray rim, repeating twice for each face; then repeat the procedure with the second frame.
c. squirt solution ***A*** from mister into sampling tray, ensuring all debris is entrained.
d. slowly pour solution and debris through filter paper into container ***A*** (**Fig. 2**).
e. inspect filter paper and remove as per **3. f.-g.**

5. Collect sample of drone brood:

a. select frames as per **1. a.-e.**
b. sample up to 100 capped coloured drone brood if present (**Fig. 3**) by sliding cappings scratcher tines along top of worker brood cells into side of and through capped drone brood, then lift scratcher upwards to remove impaled brood from cells.

Table 2. Recommended alcohol wash sampling method



5. Collect sample of drone brood (continued):

c. dislodge brood off scratcher tines into alcohol wash container ***A***, using container neck.

d. optionally, dislodge any mites from open drone cells as per **4. b.-e.**

e. screw lid tightly onto container ***A***, and screw container ***B*** tightly on top, then separate mites as per **2. b.-g.**

f. filter sample as per **3. a.-g.** using same filter paper and bag ***A*** for same hive sample.

6. Bag and label sample:

a. on completion of same-hive sampling, remove filter paper then fold over debris to enclose them by folding in half then half again.

b. place folded filter paper(s) from the same hive separately into the plastic bag ***A***.

c. seal bag and using texta, record date, brand, apiary, location and hive No. on bag and hive.

d. complete *Notification of detection/suspected detection of Varroa mites* form and place in separate plastic bag ***A***.

e. submit samples and form to PIRSA Apiary Unit.

Additional separate samples:

Clusters of bees in extraction plant formed from incompletely cleared supers could also be sampled.

Action where mites detected/suspected:

If mites/mite like insects are detected/suspected:

- contact PIRSA Apiary Unit immediately to discuss symptoms, actions and sample submission.
- if departing the apiary (eg. Inspector unable to attend in time), prevent potential spread by:
  - preventing robbing/swarming at the apiary.
  - not moving bees or equipment from apiary (incl. hives/supers of honey - even if already loaded).
  - decontaminating prior to departure (incl. leaving overalls/gloves/equipment in bags at apiary and killing/ removing bees on clothes/in cab/on tray/etc).
  - proceeding directly to home (without visiting other apiaries/apiarists).
  - decontaminating on arrival home (eg. cleaning tray/equipment with hot water and/or detergent, spraying cab, etc with fly spray, washing clothes in hot water and detergent, and showering.

Parasitic Mite Syndrome (PMS)

Adult Symptoms (can occur at any time of year):

- reduction in adult bee population.
- bees with stunted/distorted abdomens or wings and/or legs (**Fig. 4**); or crawling on ground around hive.
- unexplained repeated queen supersedure.

Brood Symptoms (can occur at any time of year):

- spotty brood pattern and/or scattered dead brood - mostly older larvae-pupae.
- symptoms similar to EFB/AFB/Sacbrood (eg. twisted in cell, "molten" to bottom of cell, light brown/yellow in colour; but no ropiness/odour present, and scales easy to remove (**Fig. 5**).
- sometimes cells uncapped (**Fig. 6**), or pupae cannibalised.
- white faecal spots on brood cells towards the back on the top wall of the cells (**Fig. 7**).
- sometimes mites (adult mites are reddish brown (**Fig. 8**), immature mites are white), on affected brood.

Fig. 1

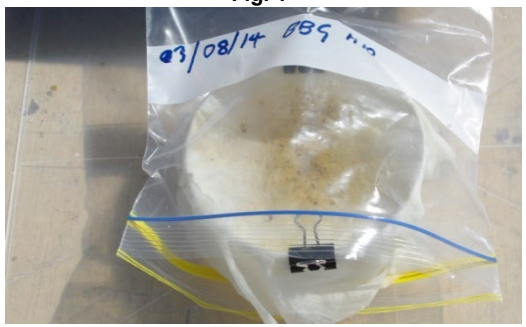


Fig. 2

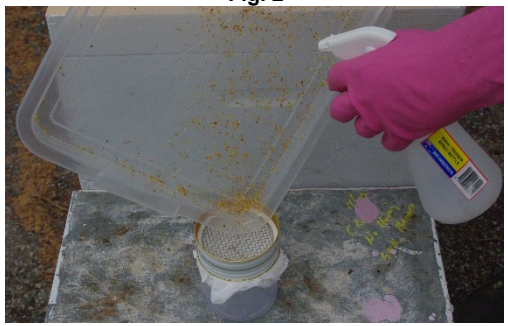


Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8