Australian Government
Australian Pesticides and Veterinary Medicines Authority

PERMIT TO ALLOW SUPPLY AND EMERGENCY USE OF UNREGISTERED AGVET CHEMICAL PRODUCTS FOR DIAGNOSIS OF MITES IN HONEYBEES

PERMIT NUMBER – PER92621

This permit is issued to the Permit Holder in response to an application granted by the APVMA under section 112 of the Agvet Codes of the jurisdictions set out below. This permit allows any Supplier to possess the product for the purposes of supply, and to supply the product to a person who can use the product under permit. This permit also allows a person, as stipulated below, to possess and use the product in the manner specified in this permit in the designated jurisdictions. This permit also allows the Permit Holder and any person stipulated below to claim that the product can be used in the manner specified in this permit.

THIS PERMIT IS IN FORCE FROM 6 JULY 2022 TO 31 JULY 2032

Permit Holder:
NSW DEPARTMENT OF PRIMARY INDUSTRIES
105 Prince Street
ORANGE, NSW 2800

Persons who can use the product under this permit:
Persons generally.

CONDITIONS OF USE

Products to be used:
ICING SUGAR
AN UNREGISTERED PRODUCT
Containing: 100 % CANE SUGAR as the only active constituent.

ISOPROPYL ALCOHOL
AN UNREGISTERED PRODUCT
Containing: ≥ 70 % ISOPROPYL ALCOHOL as the only active constituent.

METHYLATED SPIRITS
AN UNREGISTERED PRODUCT
Containing: ≥ 70% ETHYL ALCOHOL as the only active constituent.

Directions for Use:

Situation	Purpose	Rate
Surveillance and diagnosis of parasitic honey bee mites in hives	Detection of parasitic honey bee mites (including *Varroa destructor*) on European Honey Bee (*Apis mellifera*)	1 tablespoon of icing sugar per container (see **Attachment 1** for further directions)
		100 mL isopropyl alcohol or methylated spirits per container (see **Attachment 2** for further directions)

Critical Use Comments:
- Ensure that bystanders are protected from bee attack by moving them away during handling or applying at a time when bystanders are not in the vicinity.
- Refer to further directions for the relevant procedure in **Attachments 1** or **2**.

Jurisdiction:
All States and Territories.

Additional Conditions:
This permit allows for the use of a product in a manner specified on the permit. Persons who wish to prepare for use and/or use products for the purposes specified in this permit must read, or have read to them, the details and conditions of this permit. Unless otherwise stated, the use of the product must be in accordance with the product label.

GMP Licencing Exemption:
Compliance with 61(3A) of *Agricultural and Veterinary Chemicals Code Regulations 1995*, and Part 2, section 5 of the *Agricultural and Veterinary Chemicals Code (Manufacturing Principles) Determination 2014* is not required for use of the unregistered products under this permit.

Issued by the Australian Pesticides and Veterinary Medicines Authority
Note: 19/07/2022 – Permit updated to extend use in all jurisdictions.

Sugar shaking

BACKGROUND

Sugar shaking of honey bees is a passive method to detect external parasites such as Varroa mites, Tropilaelaps mites and Braula fly. It is a quick and easy method does not kill the honey bees sampled. The method works by the fine sugar particles dislodging external parasites by stopping their sticky pads (feet) gripping onto honey bees and also by stimulating grooming behaviour of honey bees. The sugar is then separated from the bees and inspected for external parasites. The efficiency of this surveillance method increases the warmer the atmospheric temperature. This method will not detect very low infestations of external parasites in hives, and is not as effective as the alcohol wash method.

Equipment required

- Jar (preferably plastic) about 500 – 750 grams in size and lid with holes 3-5 mm in size (drilled or use 1/8 gauze wire mesh)
- Pure icing sugar
- Cup (about 250 mL)
- Tablespoon
- Newspaper or large plastic sheet
- Container to hold water (e.g. small white bucket) or white sheet of paper/cardboard
- Protective clothing, smoker and hive tool
- Magnifying lens (if available)
- Filter paper (e.g. coffee filter) or fine sieve

Sugar-shake jar, pure icing sugar and measuring spoon. Image courtesy of Daniel Martin, VIC DEPI.

Procedure

- If using a container or bucket to collect sugar, half fill with water before commencing the sugar shake.
- Place 1 tablespoon of icing sugar into the jar.
- Place a large sheet of newspaper or plastic beside the hive to be tested.
- Light a smoker, open the hive and remove a frame from near the centre of the brood. If possible, take adult bees from at least 3 brood frames. If the queen is present place her back in the hive.
- Shake the bees off the frame onto the newspaper/plastic sheet and pour about 300 bees (1/2 a cup) into the jar.

Honey bees are poured into a jar for sugar shaking. Image courtesy of NSW DPI.

- Put the lid on the jar quickly to prevent the bees from escaping.
- Roll and gently shake the jar for 2-3 minutes, ensuring the honey bees are covered in sugar. Be careful not to lose any sugar. Do the shaking in a sheltered position protected from wind, so any mites present do not blow away.

Attachment 1 – Sugar Shaking Directions

- Leave for 2-3 minutes before rolling and shaking again for another 2-3 minutes. The longer the bees are rolled in the sugar, the more effective the technique.
- Shake the sugar out of the jar through the holes/mesh into a container/bucket half filled with water or shake onto a white sheet of paper/cardboard.
- Release the bees from the jar onto the ground at the hive entrance in case queen is present and inspect the empty jar thoroughly for mites.

Honey bees coated in icing sugar are returned to the hive entrance after sugar shaking. Image courtesy of Randy Oliver, www.scientificbeekeeping.com.au

- If the sugar was shaken into a bucket or container of water then the sugar will dissolve and any Varroa mites will float on the surface. Inspect the surface thoroughly for mites. A magnifying lens can be used if available.
- Alternatively the water can be gently stirred to dissolve all the sugar and then passed through filter paper (e.g. coffee filter) which can then be thoroughly inspected for Varroa mites.
- If the sugar was shaken onto a white sheet of paper or cardboard, the sugar needs to be spread finely across the paper to ensure any Varroa mites that are present are not covered with sugar particles (as below). Inspect thoroughly for Varroa mites.

Inspect white piece of paper / cardboard for mites. Image courtesy of Randy Oliver, www.scientificbeekeeping.com.au

- Alternatively the sugar can be poured through a very fine sieve that will capture the Varroa mites while allowing the sugar to pass through. The sieve contents can then be thoroughly inspected on a sheet of white paper. Note that wind can be a major problem for this particular technique.

Reporting

If Varroa mites, Tropilaelaps mites or Braula fly are suspected, report the finding immediately to the relevant state/territory agriculture agency through the **Exotic Plant Pest Hotline (1800 084 881)** or by directly reporting to the state/territory Chief Plant Health Manager.

Alcohol washing

BACKGROUND

Alcohol washing is a quick and effectively method for detecting the presence of Varroa mites, as well as monitoring colony mite levels. The disadvantage of this method is that it kills the bees that are sampled. The alcohol wash method can remove 70-80% of external Varroa mites present on adult honey bees. The method can also be used for Tropilaelaps mites and Braula fly surveillance, however, with less confidence because these pests are not present in large numbers of worker bees.

It is recommended that all beekeepers conduct this monitoring technique as it is rapid and simple. Very little equipment is required and it can be easily performed during routine hive inspections. This technique is more effective when little brood is present, however it will provide measurable results when there is also significant quantities of brood and the sample bees are taken from the centre of the brood nest.

Equipment required

- 2 plastic jars, about 500 grams in size and with wide mouth
- 3mm gauze wire mesh
- Soldering gun
- Cup (about 250 mL)
- 100 mL of 25% rubbing alcohol or 25% methylated spirits
- Newspaper or large plastic sheet
- Protective clothing, smoker and hive tool
- Magnifying lens (if available)
- A container (e.g. small white bucket)
- Filter paper (e.g. cloth, coffee filter)

How to make an alcohol washing kit

- Acquire two identical plastic jars (such as large peanut butter jars) with screw top lids.
- Carefully remove (by safely cutting) the inner section of the closed-end of the screw-top lids to acquire the desired effect.
- Cut out a 3mm gauze wire mesh and place between the two open screw-top lids.

A plastic lid with the inner removed and covered with a section of 1/8" wire mesh. Image courtesy Randy Oliver, www.scientificbeekeeping.com.

- Plastic jar lids are commonly made from polypropylene and cannot be glued effectively. Therefore, you will need to heat weld them together. A soldering gun is best suited to this purpose. A deep weld is required as a light weld may crack with use.

Attachment 2 – Alcohol Washing Directions

A soldering gun joining the two plastic lids together, with both inner lids removed and the 1/8" wire mesh sandwiched between the lids. Image courtesy Randy Oliver, www.scientificbeekeeping.com.

- Connect both of the jars to the lids and make sure that the lid connection is strong.
- You now have a functional mite shaker for conducting the alcohol washing test.

A functional mite washing kit, including a white bucket, mite shaker, bottle of 25% alcohol, a measuring cup and a sieve. Image courtesy Randy Oliver, www.scientificbeekeeping.com.

Procedure #1

- Place about 100 mL of alcohol in one of the jars, or enough so that the bees will be covered.
- Place a large sheet of newspaper or plastic beside the hive to be tested.
- Light a smoker, open the hive and remove a frame which contains a lot of brood. If the queen is present place her back in the hive.
- Shake bees from a brood frame onto the newspaper/plastic sheet/plastic tub.
- The field bees will mostly fly out of the tub immediately, leaving behind predominantly nurse bees which are likely to carry a greater quantity of phoretic Varroa mites.

Shake a brood comb into a container. Image courtesy Daniel Martin, VIC DEPI.

- Pour about 300 bees (1/2 a cup) into the jar containing around 100ml of 25% rubbing alcohol or methylated spirits.

Attachment 2 – Alcohol Washing Directions

Pouring bees into the mite shaker. Image courtesy Daniel Martin, VIC DEPI.

Shake the mite shaker jars vigorously for around 20 seconds. Image courtesy Daniel Martin, VIC DEPI.

- Put the solid lid on the jar quickly to prevent the bees from escaping.

Screw the two mite shakers together firmly. Image courtesy Daniel Martin, VIC DEPI.

- Invert the shaker so that the bees are now in the top jar.
- Shake the jar vigorously for 20 seconds, ensuring the honey bees are covered in alcohol. It is essential to maintain a vigorous shaking motion in order for the alcohol to swirl with the bees in the top jar.
- After 20 seconds and the last shake, jiggle the jar so that the alcohol drains through the bees into the bottom jar. If you don't jiggle, some of the mites may get stuck on the bees in the top jar.
- Once settled, raise the bottom of the jar to view any mites that have been dislodged from the bees.

A large number of Varroa mites present. The blue arrows indicate some at the bottom of the mite shaker after settling. Image courtesy Daniel Martin, VIC DEPI.

Procedure #2

- This method does not require a soldering gun to join plastic jars together.
- Get a plastic jar with an intact covered lid.
- Follow the first 6 steps as outlined in Procedure #1.
- Shake the bees in the jar with the alcohol for 20 seconds.
- Place a piece of cloth over the top of a small bucket or container, with the 3mm gauze wire mesh slightly over the cloth.
- Pour the contents of the jar through the mesh and over the cloth. The mesh lid will collect the bees but enable any mites to pass through and be collected on the cloth.
- Inspect the surface of the cloth thoroughly for mites. A magnifying lens can be used if available.
- Higher recovery rates of Varroa mite can be achieved by refilling the jar containing bees with water and rinsing the bees once or twice. Two rinses will recover more than 95% of Varroa mites present on the bees.
- This method does not require a soldering gun to join jars together.

Reporting

- If Varroa mites, Tropilaelaps mites or Braula fly are suspected, report the finding immediately to the relevant state/territory agriculture agency through the **Exotic Plant Pest Hotline (1800 084 881)** or by directly reporting to the state/territory Chief Plant Health Manager.