2021

BOOK OF METHODS

PERFORMANCE TESTERS

Authors:

Aleksandar Uzunov
Ralph Büchler
Cecilia Costa
Fanny Mondet
Malgorzata Bienkowska
Fani Hatjina
Marina Meixner
Sreten Andonov
Marin Kovačić
Raffaele Dall'Olio
Benjamin Basso
Jerzy Wilde
Beata Panasiuk
Leonidas Charistos
Daniel Brechensbauer

European Commission

EU pilot study: Restructuring of the honey bee chain and Varroa resistance breeding & selection programme

AGRI-2017-0346

Technical support for formatting:
Daniel Stojanovski
Magdalena Jovanovska

This is non peer reviewed article.

To cite this article:
Uzunov et al., (2021) Book of Methods for Performance Testers, EURBEST project (AGRI-2017-0346), Bee Institute in Kirchhain, Germany (English language version).

Publisher

Edition
First pdf printing edition May 2021
www.eurbest.eu

TABLE OF CONTENTS

Name of tester	Apiary	Queen's Origin	Colony #No.

Date	No. combs with bees	No. combs with brood	Gentleness [1 to 4]	Swarming [1 to 4]	Honey harvest [kg]	Mites per 10 g/bees	Sealed cells [after 6h]	Observation and comments

! Column "Observation and comments": Here you should provide information related to disease incidents, applied treatments, production of swarms or nucleuses, number of added or taken frames and suppers, colony and/or queen losses etc.

TRAIT PT

BEE POPULATION [COLONY DEVELOPMENT]

METHOD

ESTIMATION BY NUMBER OF COMBS OCCUPIED WITH BEES

Recommended periods

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
		Mar	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**		

Conditions

Appropriate weather and beekeeping working conditions.

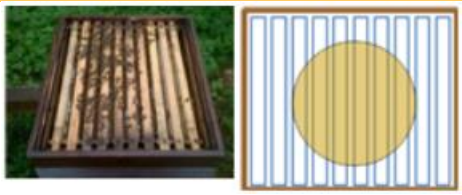
Required materials & equipment

- Standard beekeeping tools and equipment.
- Colony's recordkeeping card (please see file "PT recordkeeping card").

Size of the colony

1 TO 3 COMBS WITH BEES

4 TO 5 COMBS WITH BEES



6 TO 7 COMBS WITH BEES

8 TO 10 COMBS WITH BEES

!

Combs occupied at least 70% with bees can be considered as one.

Intermediate values (0.5 frames covered with bees) can be used to describe slight differences between the colonies.

Weather conditions can significantly affect estimation. During cold weather bees form a tighter cluster and in warm weather bees are more dispersed around the hive. Please always note your observations.

Procedure

1

Open the colony from above and without (or limited) use of smoke estimate the number of combs occupied with bees (see the criteria example above).

2

Check the same box looking from below and calculate the average value for the box.
Example:

(No. of combs occupied with bees from top + No. of combs occupied with bees from the bottom)/2 = Average no. of combs occupied with bees from the box.

3

For colonies with several boxes check all the boxes from above and below and sum up their averages.
Example (colony with 3 boxes):

(average of box 1) + (average of box 2) + (average of box 3) = Number of combs occupied with bees in the colony

4

Record the value on the colony's "PT recordkeeping card" under the column:
No. of combs with bees

Name of tester		Apiary			Queen's Origin		Colony #No.

Date	No. combs with bees	No. combs with brood	Gentleness [1 to 4]	Swarming [1 to 4]	Honey harvest [kg]	Mites per 10 g/bees	Sealed cells [after 6h]	Observation and comments

! Column "Observation and comments": Here you should provide information related to disease incidents, applied treatments, production of swarms or nucleuses, number of added or taken frames and suppers, colony and/or queen losses etc.

Notes & Suggestions

- There should be at least 3 inspections during the study: before wintering 2019, early spring 2020 and summer 2020.
- In the case of assessing a population with expressed high defensive behaviour, an alternative estimation only from the top of the box should be used (the same approach and criteria must be used for all colonies in the apiary).
- Discuss the size of the colony with your working partner.
- All colonies within one apiary need to be inspected on the same day.
- Combine the inspection of the bee population with observations or tests of the remaining traits/parameters (for instance “Gentleness” etc.).
- Take precautionary measures to prevent the possibility of robbing behaviour between the colonies.

Additional information

Standard methods for rearing and selection of *Apis mellifera* queens (Büchler *et al.*, 2013)

Virtual apiary (www.smartbees-fp7.eu/extension)

Selektion der Honigbiene (IWF Wissen und MediengGmbH, Nonnenstieg 72 D-37075 Göttingen) Video material

TRAIT PT

BROOD AREA [COLONY DEVELOPMENT]

METHOD

ESTIMATION BY NUMBER OF COMBS WITH BROOD

Recommended periods

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec

Conditions

Appropriate weather and beekeeping working conditions.

Required materials & equipment

- Standard beekeeping tools and equipment.
- Colony's recordkeeping card (please see file "PT recordkeeping card").

Procedure

1

Inspect all combs from the colony (all boxes with brood) and count the number of combs with brood (open and sealed brood).

2

Record the value for No. of combs with brood on the colony's "PT recordkeeping card"

Name of tester	Apiary	Queen's Origin	Colony #No.

Date	No. combs with bees	No. combs with brood	Gentleness [1 to 4]	Swarming [1 to 4]	Honey harvest [kg]	Mites per 10 g/bees	Sealed cells [after 6h]	Observation and comments

! Column "Observation and comments": Here you should provide information related to disease incidents, applied treatments, production of swarms or nucleuses, number of added or taken frames and suppers, colony and/or queen losses etc.

Notes & Suggestions

- There should be at least 4 inspections during the study: before wintering 2019, early spring 2020 and summer 2020, 6 weeks after the “early spring” inspection and at the end of the study (summer 2020).
- Even a small area the size of a tennis ball with open and/or sealed brood is considered a brood comb.
- Brood area found only on one side of the comb, counts as 0.5 comb.
- All colonies within one apiary need to be inspected on the same day.
- Combine the inspection of amount of brood with observations or tests of the remaining traits/parameters (for instance “Bee population” etc.).
- Take precautionary measures to prevent the possibility of robbing behaviour between the colonies.

Additional information

Standard methods for rearing and selection of *Apis mellifera* queens (Büchler *et al.*, 2013)

Virtual apiary (www.smartbees-fp7.eu/extension)

Selektion der Honigbiene (IWF Wissen und MediengGmbH, Nonnenstieg 72 D-37075 Göttingen) Video material

TRAIT PT

HONEY PRODUCTION [HONEY YIELD]

METHOD

WEIGHTING NET HONEY PRODUCTION PER COLONY

Recommended periods

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec

Conditions

During the honey production season.

Required materials & equipment

- Standard beekeeping tools and equipment.
- Balance with a precision of 0.1kg for weighting (Fig. 1).
- Colony's recordkeeping card (please see file "PT recordkeeping card").

Fig. 1

Procedure

The suppers filled with honey combs are weighted before (Fig. 2) and after extracting (Fig. 3) the difference is recorded as the honey harvest for each colony.

Fig. 2

Fig. 3

1

Record the date of each extraction in the “PT recordkeeping card”.

Honey stored in the brood nest is not considered part of the honey production.

All honey harvested within the season from an individual hive, is recognized as total honey production for the test colony. A potential crop from swarms or permanent splits, coming from the test colony, is not regarded.

The standard tare method can be used if all supers are of identical shape and with newly built combs.

2

Record the value on the colony’s “PT recordkeeping card” under the column:
Honey harvest [kg]

Name of tester		Apiary		Queen’s Origin		Colony #No.		
Date	No. combs with bees	No. combs with brood	Gentleness [1 to 4]	Swarming [1 to 4]	Honey harvest [kg]	Mites per 10 g/bees	Sealed cells [after 6h]	Observation and comments

! Column “Observation and comments”: Here you should provide information related to disease incidents, applied treatments, production of swarms or nucleuses, number of added or taken frames and suppers, colony and/or queen losses etc.

Notes & Suggestions

- The honey harvest of different time periods should be reported separately in order to document the colony’s development and adaptability to exploit different harvests
- For accurate calculations of honey production note any supplementary feeding or removal and/or placement of combs of food in each colony

Additional information

Standard methods for rearing and selection of *Apis mellifera* queens (Büchler *et al.*, 2013)

Miscellaneous standard methods for *Apis mellifera* research (Human *et al.,* 2013)

A Europe-wide experiment for assessing the impact of genotype-environment interactions on the vitality and performance of honey bee colonies: Experimental design and trait evaluation (Costa *et al.*, 2012)

Virtual apiary (www.smartbees-fp7.eu/extension)

Selektion der Honigbiene (IWF Wissen und MediengGmbH, Nonnenstieg 72 D-37075 Göttingen) Video material

TRAIT PT

SWARMING BEHAVIOUR

METHOD

CLASSIFICATION USING A STANDARD SCALE [SCORE OF 1 TO 4]

Recommended periods

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec

Conditions

During the active swarming season.

Required materials & equipment

- Standard beekeeping tools and equipment.
- Colony's recordkeeping card (please see file "PT recordkeeping card").

Standard Scale

SCORE OF 1	SCORE OF 2
Active swarming: the test colony swarmed or swarming could be prevented only by the strong intervention (interim nucleus etc.).	Strong swarming tendency as indicated by repeated queen cell construction and advanced symptoms of preparation for swarming (reduction of open brood, emaciated queen, limited comb construction).
SCORE OF 3	**SCORE OF 4**
Low swarming tendency: some queen cells with brood are present, but the overall colony condition does not indicate immediate swarming activities. The preparation for swarming maybe stopped by destroying the swarm cells and offering additional comb space.	The colony does not show any swarming tendency. There are no swarm cells containing eggs, larvae or pupae.

Procedure

1

a **Extended procedure:** inspect every comb in the colony as usual during the regular inspection for other traits.

b **Quick procedure:** check the colonies frequently, during the active swarming season or when swarming was previously observed, for the presence of cells from the bottom of each brood box (Fig. 1 and Fig. 2).

Fig. 1

Fig. 2

2

Record the score on the colony's "PT recordkeeping card" under the column:
Swarming [1 to 4]

Name of tester		Apiary			Queen's Origin		Colony #No.

Date	No. combs with bees	No. combs with brood	Gentleness [1 to 4]	Swarming [1 to 4]	Honey harvest [kg]	Mites per 10 g/bees	Sealed cells [after 6h]	Observation and comments

! Column "Observation and comments": Here you should provide information related to disease incidents, applied treatments, production of swarms or nucleuses, number of added or taken frames and suppers, colony and/or queen losses etc.

Notes & Suggestions

- Check the colonies frequently (every 7 - 9 days) where swarming behaviour was previously observed.
- For better observation (during the quick procedure) use smoke to push bees higher into the box.
- Combine the inspection of bee population with observations or tests of the remaining traits/parameters (for instance "Bee population", "Brood area" etc).
- After each inspection note your observation (score 1 to 4) on the "PT recordkeeping card".
- The supersedure queen cells are not considered as a swarm cell.

Additional information

Standard methods for rearing and selection of *Apis mellifera* queens (Büchler *et al.*, 2013)

A Europe-wide experiment for assessing the impact of genotype-environment interactions on the vitality and performance of honey bee colonies: Experimental design and trait evaluation (Costa *et al.*, 2012)

Virtual apiary (www.smartbees-fp7.eu/extension)

Selektion der Honigbiene (IWF Wissen und MediengGmbH, Nonnenstieg 72 D-37075 Göttingen) Video material

TRAIT PT

GENTLENESS [DEFENSIVE BEHAVIOUR]

METHOD

CLASSIFICATION USING A STANDARD SCALE [SCORE OF 1 TO 4]

Recommended periods

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec

Conditions

Appropriate weather and beekeeping working conditions.

Required materials & equipment

- Standard beekeeping tools and equipment.
- Colony's recordkeeping card (please see file "PT recordkeeping card").

Standard Scale

SCORE OF 1

In spite of the use of smoke the colony shows a strong defensive reaction on being handled, or bees attack without being disturbed.

SCORE OF 2

Single bees attack and sting during the working procedure, even if smoke is used intensively.

SCORE OF 3

The colony can easily be worked without stings, if using some smoke.

SCORE OF 4

No use of smoke and no protective clothes are necessary to avoid stings during the normal working procedure.

! An intermediate score (0.5 of a point) can be used to better describe slight differences between the colonies.

Procedure

1 Inspect the colony in a regular way and if feasible reduce the use of smoke as much as possible.

2 Observe the bees' behaviour during the inspection.

3 Discuss bees' behaviour with your working partner.

4 Record the score on the colony's "PT recordkeeping card" under the column:
Gentleness [1 to 4]

Name of tester		Apiary			Queen's Origin		Colony #No.

Date	No. combs with bees	No. combs with brood	Gentleness [1 to 4]	Swarming [1 to 4]	Honey harvest [kg]	Mites per 10 g/bees	Sealed cells [after 6h]	Observation and comments

! Column "Observation and comments": Here you should provide information related to disease incidents, applied treatments, production of swarms or nucleuses, number of added or taken frames and suppers, colony and/or queen losses etc.

Notes & Suggestions

- All colonies within one apiary need to be inspected on the same day.
- Perform colony inspections under any weather conditions which you would normally inspect colonies.
- If possible, start the inspection without smoke, but if it is not feasible then use limited amount of smoke on the hive entry before the inspection. It is vital to maintain the same criteria for all colonies in the apiary.
- Due to the influence of neighbouring colonies, the order of colonies' inspection should be different for each testing day.
- Change the order of the colonies' inspection if neighbouring colonies are disturbed as a consequence of their inspection (change the order, but still complete them in a single day).

- If the colony is heavily disturbed, for instance as a consequence of a fallen hive tool, frame etc., consider the condition and adjust the final score.
- Combine the inspection of gentleness with observations or tests of the Remaining traits/parameters (for instance “Bee population”, “Brood area” etc).
- Lack of nectar flow can strongly interfere with colony inspection and testing due to incidents of robbery between the colonies and resulting in the expression of defensiveness.
- Take precautionary measures to prevent the possibility of robbing behaviour between the colonies.

Additional information

Standard methods for rearing and selection of *Apis mellifera* queens (Büchler *et al.*, 2013)

A Europe-wide experiment for assessing the impact of genotype-environment interactions on the vitality and performance of honey bee colonies: Experimental design and trait evaluation (Costa *et al.*, 2012)

Virtual apiary (www.smartbees-fp7.eu/extension)

Selektion der Honigbiene (IWF Wissen und MediengGmbH, Nonnenstieg 72 D-37075 Göttingen) Video material

TRAIT PT

ADULT BEE INFESTATION [VARROA]

METHOD

POWDERED [ICING] SUGAR

Recommended periods

Jan	Feb	Mar	Apr	May	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	Nov	Dec

Conditions

Dry and calm weather conditions.

Required materials & equipment

- Clear plastic sheet with a minimum size of 40x40 cm (Fig. 1).
- Jar or sample tube with a minimum size of 120 ml (Fig. 2).
- Jar for shaking (minimum size of 750 ml, for instance 1 kg- yogurt tub with lid) with fixed metal mesh (size 2.8 mm) on the cut bottom by heating (Fig. 3).
- Icing sugar. Use a new packet containing dry sugar, ca. 250 g for 7 colonies.
- Tablespoon.
- Very fine sieve (Fig. 4).
- Bright coloured bucket, for instance honey bucket.
- Kitchen balance/weighing scale.
- Record sheet for the method Powdered (Icing) sugar (Annex 1).
- Colony's recordkeeping card (please see file "PT recordkeeping card").

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Procedure

1

STEP 1

Open the hive's lid and from the outer frame of the top box shake about 50g bees (≈500 worker bees) onto the sheet.

STEP 2

Fold the sheet and dislodge the bees in to the sample tube. Working quickly weight the bees.

STEP 3

Dislodge the bees from the cup into the jar for shaking, and turn the jar upside down with the mesh on top.

STEP 4

Add 5 tablespoons of powdered sugar and carefully shake the jar for complete sugar distribution throughout the bees.

STEP 5

Leave the jar for 3 minutes with the mesh bottom-up and from time to time shake it.

STEP 6

Invert the jar and shake for around 1 minute so that the powdered sugar and the mites pass through the mesh.

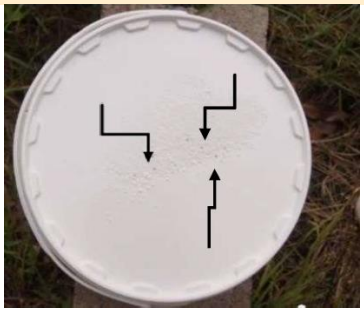
STEP 7

Replace the powdered bees into the colony.

STEP 8

Shake the powdered sugar through the fine sieve leaving the mites behind.

STEP 9



Dislodge and count the mites on a bright surface. Record the value.

2

For calculation of the infestation level expressed as number of mites in 10g of bees use the following formula:

$$\frac{\textbf{Total number of mites} * \textbf{10}}{\textbf{Bees net weight (g)}} = \textbf{mites per 10g bees}$$

3

Record and calculate the value on the Record Sheet for the Powdered sugar method (Annex 1).

4

Once complete record the date and final value on the colony's "PT recordkeeping card" under the column: **Mites per 10g/bees**

Name of tester	Apiary	Queen's Origin	Colony #No.

Date	No. combs with bees	No. combs with brood	Gentleness [1 to 4]	Swarming [1 to 4]	Honey harvest [kg]	Mites per 10 g/bees	Sealed cells [after 6h]	Observation and comments

! Column "Observation and comments": Here you should provide information related to disease incidents, applied treatments, production of swarms or nucleuses, number of added or taken frames and suppers, colony and/or queen losses etc.

Notes & Suggestions

- Assess the adult bee infestation (Varroa) at least 3 times in the season (consult with EURBEST national coordinator).
- During windy conditions use a shelter.

Additional information

www.youtube.com/watch?v=-ZQmm78nMnE - Varroa Befallskontrolle mit Puderzucker

Standard methods for rearing and selection of *Apis mellifera* queens (Büchler *et al.*, 2013)

Standard methods for varroa research (Dietemann *et al.,* 2013)

Virtual apiary (www.smartbees-fp7.eu/extension)

Annex 1 [Record Sheet]

POWDERED SUGAR METHOD Varroa infestation			Date	Location/Apiary	Breeder/Tester
No.	Colony's identification number	Bee net weight (g) [A]	Total number of mites [B]	Mites per 10g of bees (≈ % of infestation) [B*10] / A	Notes
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

TRAIT PT

ADULT BEE INFESTATION [VARROA]

METHOD

WASHING METHOD [ALTERNATIVE]

Recommended periods

Jan	Feb	Mar	Apr	May	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	Nov	Dec

Conditions

Appropriate weather, beekeeping and lab working conditions.

Required materials & equipment

- Clear plastic sheet with a minimum size of 40x40 cm (Fig. 1).
- Jar or sample tube with minimum a size of 120 ml (Fig. 2).
- Jar for shaking (minimum size of 750 ml, Fig. 3).
- 60 ml of dish-washing detergent for 1-litre water.
- Water supply with strong and dispersed water jet (Fig. 4).
- One sieve to detain the bees and another very fine sieve to detain the mites (Fig. 4).
- Kitchen balance/weighing scale.
- Record sheet for the method Washing method (Annex 1).
- Colony's recordkeeping card (please see file "PT recordkeeping card").

Fig. 1

Fig. 2

Fig. 3

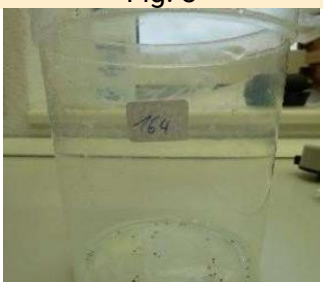


Fig. 4

Procedure

1

STEP 1

Open the hive's lid and from the outer frame of the top box shake about 50g bees (≈500 worker bees) onto the sheet.

STEP 2

Fold the sheet and dislodge the bees in to the sample tube. Working quickly weight the bees.

STEP 3

Dislodge the bees into the jar with soupy water above the bees.

STEP 4

Leave the jar for 30 minutes and occasionally shake the content.

STEP 5

Dislodge the bees into the sieve and wash off with water jet. Separate the mites from the bees by use of a double sieve (lower fine).

STEP 6

Count the separated mites and do the calculation.

2

For calculation of the infestation level expressed as a number of mites in 10g of bees use the following formula:

$$\frac{\textbf{Total number of mites} * \textbf{10}}{\textbf{Bees net weight (g)}} = \textbf{mites per 10g bees}$$

3

Record and calculate the value on the Record Sheet for Washing method (Annex 1).

4

Once complete record the date and final value on the colony's "PT recordkeeping card" under the column: **Mites per 10g/bees**

Name of tester		Apiary		Queen's Origin		Colony #No.		
Date	No. combs with bees	No. combs with brood	Gentleness [1 to 4]	Swarming [1 to 4]	Honey harvest [kg]	Mites per 10 g/bees	Sealed cells [after 6h]	Observation and comments

! Column "Observation and comments": Here you should provide information related to disease incidents, applied treatments, production of swarms or nucleuses, number of added or taken frames and suppers, colony and/or queen losses etc.

Notes & Suggestions

Asses the adult bee infestation (Varroa) at least 3 times in a season (consult with EURBEST national coordinator) .

Additional information

www.youtube.com/watch?v=-ZQmm78nMnE - Varroa Befallskontrolle mit Puderzucker

Standard methods for rearing and selection of *Apis mellifera* queens (Büchler *et al.*, 2013)

Standard methods for varroa research (Dietemann *et al.,* 2013)

Virtual apiary (www.smartbees-fp7.eu/extension)

Annex 1 [Record Sheet]

WASHING METHOD Varroa infestation			Date	Location/Apiary	Breeder/Tester
No.	Colony's identification number	Bee net weight (g) [A]	Total number of mites [B]	Mites per 10g of bees (≈ % of infestation) [B*10] / A	Notes
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

TRAIT PT

NATURAL VARROA MITE MORTALITY [OPTIONAL]

METHOD

NATURAL MITEFALL

Recommended periods

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
	Feb	**Mar**	**Apr**								

Conditions

Early spring during the main spring pollen production.
Carried out during periods without any Varroa-treatment.

Required materials & equipment

- Screened bottom board (Fig. 1).
- Sticky white pattern sheets (Fig. 2) or the use of oil (Fig. 3).
- Record sheet for Natural mite fall (Annex 1).
- Colony's recordkeeping card (please see file "PT recordkeeping card").

Fig. 1

Fig. 2

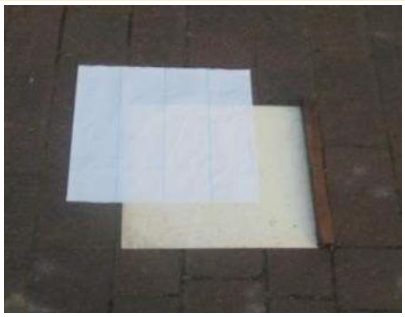


Fig. 3

Procedure

1

STEP 1

Place the sticky (or oily) white pattern sheet on the bottom board and leave it for 7 days.

STEP 2

After 7 days count the fallen mites from the sticky (oily) sheets, but also check for mites on the fallen dead bees.
Record the values.

STEP 3

Clean the sheet or use a new one and leave it for another 7 days.

2 Collect data every few days (about one a week) and record on the Record sheet for Natural mite fall (Annex 1).

3 Repeat the method for 3 consecutive weeks.

4 Sum the values from the whole control period and divide by the number of days (about 21).

5 Check the same box looking from below and calculate the average value for the box.
Example:

1st week = 8 fallen mites
2nd week = 3 fallen mites
3rd week = 11 fallen mites

$$\frac{\textbf{Total 22 fallen mites}}{\textbf{21 days (3 weeks)}} = \textbf{1.05 mites/day}$$

6 After 3 weeks record the date and final value on the colony's "PT recordkeeping card" under the column: Observation and comments (OPTIONAL)

Name of tester		Apiary		Queen's Origin				Colony #No.
Date	No. combs with bees	No. combs with brood	Gentleness [1 to 4]	Swarming [1 to 4]	Honey harvest [kg]	Mites per 10 g/bees	Sealed cells [after 6h]	Observation and comments

! Column "Observation and comments": Here you should provide information related to disease incidents, applied treatments, production of swarms or nucleuses, number of added or taken frames and suppers, colony and/or queen losses etc.

Notes & Suggestions

- Prevent the presence of ants (use of sticky sheets or oil).
- During windy conditions use a shelter.

Additional information

Standard methods for rearing and selection of *Apis mellifera* queens (Büchler *et al.*, 2013)

Miscellaneous standard methods for *Apis mellifera* research (Human *et al.,* 2013)

A Europe-wide experiment for assessing the impact of genotype-environment interactions on the vitality and performance of honey bee colonies: Experimental design and trait evaluation (Costa *et al.*, 2012)

Virtual apiary (www.smartbees-fp7.eu/extension)

Selektion der Honigbiene (IWF Wissen und MediengGmbH, Nonnenstieg 72 D-37075 Göttingen) Video material

Annex 1 [Record Sheet]

NATURAL MITE FALL Natural mite mortality			Date of first sheet insertion		Location/Apiary	Breeder/Tester	
No.	Colony's identification number	Number of fallen mites after the first week [A]	Number of fallen mites after the second week [B]	Number of fallen mites after the third week [C]	Total number of fallen mites SUM=A+B+C	Daily mite fall SUM/21	Notes [Record date A,B,C]
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							

TRAIT PT
HYGIENIC BEHAVIOUR

METHOD
PIN TEST

Recommended periods

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec

Conditions

Dry and calm weather conditions.
Avoid periods with intensive nectar flow and pollen collection.
Additional people to assist.

Required materials & equipment

- Pin test kit:
  - Pattern (wooden, metal or plastic), 10x10 cells wide (Fig. 1).
  - Entomological pin size No.2 (Fig. 2).
- Colony's recordkeeping card (please see file "PT recordkeeping card").

Fig. 1

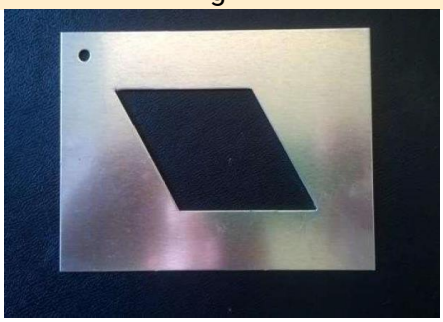
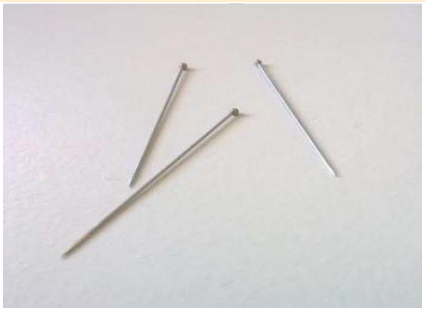


Fig. 2



Procedure

1

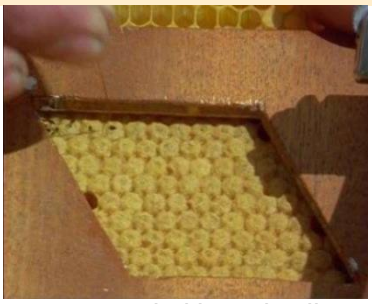
STEP 1

Find a frame with sealed worker brood, at the stage of young white or reddish eyed pupae.

STEP 2

Place the pattern (covering 100 cells) and mark the upper left (cell) and lower right (cell) with a colour felt-tip pen.

STEP 3



Pierce 50 sealed brood cells (starting from the first cell after the marked one) row by row from left to right with the entomological pin. Pierce the cap and push the pin towards the bottom until it reaches the base of the cell.

STEP 4

Mark the cell 51 with the pen to identify the treated area.

STEP 5

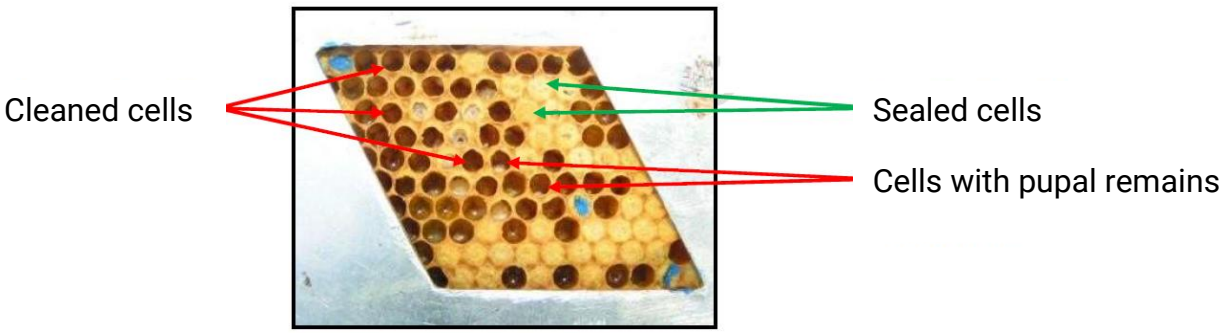
Mark the frame on the top bar and indicate the side that was treated and place back the frame in its former position.

STEP 6

Check the colony after 6 hours. Count only the sealed brood cells. Record the value.

2

During the piercing of the brood skip all empty cells and disregard them in any calculation!



3

After 3 weeks record the date and final value on the colony's "PT recordkeeping card" under the column: **Sealed cells [after 6h]**

Name of tester	Apiary	Queen's Origin	Colony #No.

Date	No. combs with bees	No. combs with brood	Gentleness [1 to 4]	Swarming [1 to 4]	Honey harvest [kg]	Mites per 10 g/bees	Sealed cells [after 6h]	Observation and comments

! Column "Observation and comments": Here you should provide information related to disease incidents, applied treatments, production of swarms or nucleuses, number of added or taken frames and suppers, colony and/or queen losses etc.

Notes & Suggestions

- Repeat the test at least 2 times in the season.
- The method should be applied not earlier than 40 days following the establishment of the colonies.

Additional information

Standard methods for rearing and selection of *Apis mellifera* queens (Büchler *et al.*, 2013)

A Europe-wide experiment for assessing the impact of genotype-environment interactions on the vitality and performance of honey bee colonies: Experimental design and trait evaluation (Costa *et al.*, 2012)

AGT methodenhandbuch 2012

Virtual apiary (www.smartbees-fp7.eu/extension)

TRAIT PT

BROOD INFESTATION, SMR & REC

METHOD

BROOD SAMPLING

Recommended periods

Jan	Feb	Mar	Apr	May	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	Nov	Dec

Conditions

Active season (consult with EURBEST national coordinator).
Carried out during periods without any Varroa-treatment.

Required materials & equipment

- Sharp knife or scalpel (disinfecting after each colony sampling).
- Plastic bag for storage and shipment of the brood sample.
- Pencil and paper.
- Colony's recordkeeping card (please see file "PT recordkeeping card").

Procedure

1. Open the colony and find a comb with sealed brood.
2. Cut sealed brood section of 20x20 cm with brood mainly containing elder pupae (pink eyes stage to moult completed, corresponding to 7-12 days post capping).
3. Record the location of the apiary, the number of the colony and the date on the paper using a pencil. Add the paper in the plastic bag together with the sampled brood.
4. The brood samples should immediately be evaluated or stored in freezers (-18°C) until the examination.
5. In case of shipment to laboratory, send the samples using dry ice box and express delivery service.

6

Once complete record the date and final value on the colony's "PT recordkeeping card" under the column: **Observation and comments**

Name of tester		Apiary		Queen's Origin		Colony #No.	

Date	No. combs with bees	No. combs with brood	Gentleness [1 to 4]	Swarming [1 to 4]	Honey harvest [kg]	Mites per 10 g/bees	Sealed cells [after 6h]	Observation and comments

! Column "Observation and comments": Here you should provide information related to disease incidents, applied treatments, production of swarms or nucleuses, number of added or taken frames and suppers, colony and/or queen losses etc.

Notes & Suggestions

The sampling colonies should have an undisturbed brood development for at least 30 days prior to sampling (no brood interruption, no queen exchange). As the efficiency of the brood analysis depends on high infestation levels the sampling date may be postponed until Varroosis treatment needs to be started. According to the experience, the bee infestation should be at least about 2 % at the time of brood sampling for SMR control.

Additional information

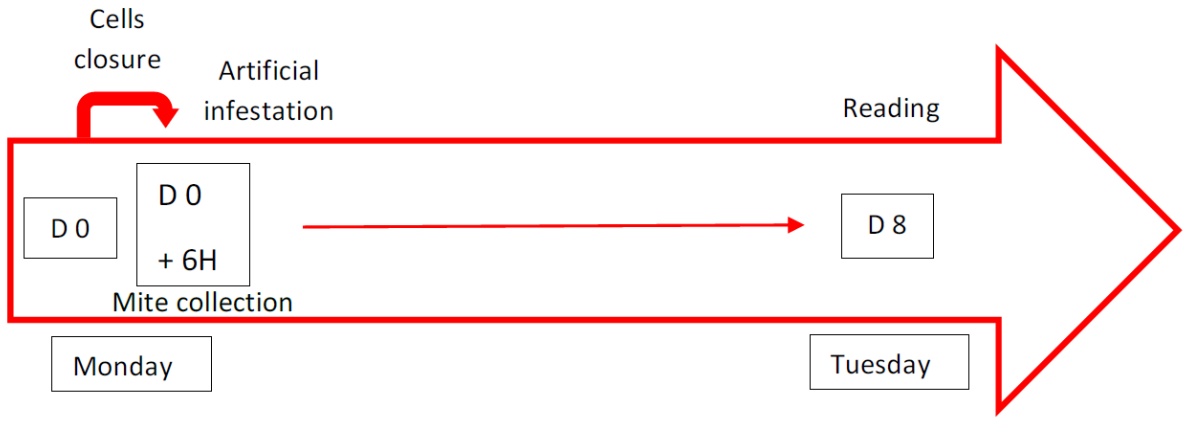
Research Network for Sustainable Bee Breeding

Screening for low Varroa mite reproduction (SMR) and recapping in European honey bees (Büchler et al., 2017)
www.beebreeding.net

TRAIT PT

VSH - ARTIFICIAL INFESTATION PROTOCOL

Timeline of the experiment



Material

- To mark cells:
  - Transparent sheets
  - Pins
  - Brush
  - Markers
- To harvest Varroa (with the sugar shake method):
  - Sugar
  - Sieve
  - Water
  - Thin Brushes
  - Petri dish
  - Filters
  - Shaker and lid with holes
- To infest cells:
  - Scalpel
  - Tweezers
  - Fine paintbrushes
  - Stands for the frames
  - Light
  - Transparent sheets with cells marked 6 hours earlier
  - Varroas (conserved in a petri dish)

METHODS

The experiment of artificial infestation requires one day to infest cells and one day, 8 days after infestation, to assess for uncapping behavior.

1

Day 0 - first step in the morning: Mark cells ready to be capped

Select a frame with cells close to capping or partially capped (L5 stage) (Figure 1).
Place the transparent sheet on the frame with pins.
Note the colony number on the upper part of the frame, the date, time and colony number on the transparent sheet (plus the frame number if more than one frame is marked).
Mark cells (Figure 2) to obtain at least 30 capped cells for infestation 6 hours later (to be safe ~100-150 cells).
Before putting the frame back into the hive, remove the transparent sheet but leave the pins on the frames until the end of the experiment.

Figure 1: Cells close to capping or partially capped (L5, L6 stage)

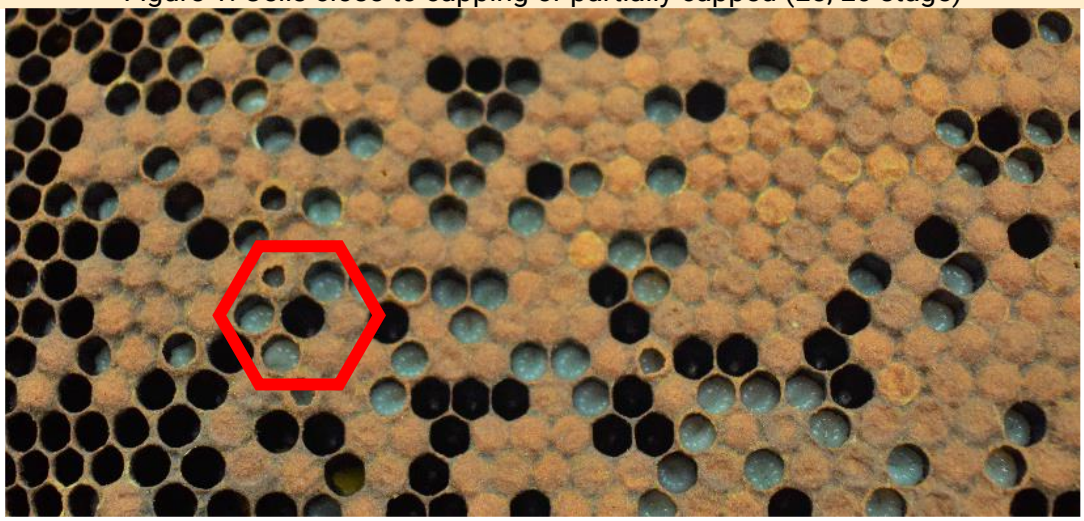


Figure 2: Frames with transparent sheet and marked cells

2

Day 0 - second step 5 to 6 hours later (early afternoon): Collect varroas

Put several hundred adult bees in the shaker with sugar (beware of the queen!).
Shake to take the varroa off the bees. Then, shake over the sieve and rinse the remaining sugar with water.
Collect varroas in the sieve with a paintbrush and put them in a petri dish on a moist filter paper.

Sugar shake method
(Dietemann, V., Nazzi, F., Martin, S. J., Anderson, D. L., Locke, B., Delaplane, K. S., ... & Rosenkranz, P. (2013). Standard methods for varroa research. Journal of apicultural research, 52(1), p 11.)

3

Day 0 - third step 5 to 6 hours later (in parallel or just after varroa collection): Artificial infestation

Place the transparent sheet on the frame(s) and verify if there are more than 30 cells freshly capped. Use the stand for the frame to be comfortable for the infestation.
Infest 30 cells with adult varroas.
Carefully open one side of the brood cell cap with a scalpel to avoid killing the larva and slip a mite inside each opened cell using a fine paintbrush (easier when the brush is wet) (Figure 3).
Delicately close the cell using the back of the paintbrush. Follow the same method for the control cells (without inserting a mite), if possible, do as many control cells as infested cells.
Infested and control cells should be well identified on the transparent sheet using two different colours.
Return the frame into the hive with the pins (remove the transparent sheet).

Figure 3: Infestation step

4

Day 8 - fourth step: Reading the results

Eight days later, place the transparent sheet on the frame to read the results of each infested and control cells (for results sheet, see figure 4, 5).
There are 2 cases observed: Opened and cleaned cells, or closed cells.
For the closed cells, use tweezers to open it and check if it is re-capped or not and note the absence/presence of *Varroa* (alive or dead) and the absence/presence of descendants.

Figure 4: Reading sheet, to be used in the field to collect data.

Hive ID:								
Date (J0):		Frame:		Experim. Infestation:				
Date (J8):				Experim. Reading:				

No. cell info	Opened and cleaned	Recapped	Untouched	Foundress			Descendants	
				Nb	Dead	Alive	Yes	No
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								
31								
32								
33								
34								
35								
36								
37								
38								
39								
40								
41								
42								
42								
43								
44								
45								