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A COMPARATIVE ANALYSIS OF SHAKING SOLUTIONS FOR THE DETECTION OF *VARROA JACOBSONI* ON ADULT HONEYBEES (1)

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SUMMARY

Various liquids could be used for the detection of *Varroa jacobsoni* on honey bees using the shaking method. Mechanical shaking for 30 minutes in a rotary shaker disloged all but 3 mites in 27 separate examinations.

The ectoparasitic honey bee mite *Varroa jacobsoni*, first described by OUDEMANS (1904) from the Asian honey bee *Apis cerana* in Java, is distributed throughout Asia, wherever *A. Cerana* occurs (DE JONG and MORSE 1979). Since the 1950's, and probably earlier, beekeepers have moved colonies of European bees, *A. mellifera*, into Asia (CRANE 1978) and in some cases colonies of *A. cerana* (infested with *Varroa*) from Asia into Europe (LANGHE and NATSKII 1976, RUTTNER and RITTER, 1980) and, as a result of contact between the two bee species, *V. jacobsoni* has moved to *A. mellifera* as a new host (CRANE 1978, MORSE, 1978). Although *A. cerana* is not seriously affected by the mites, colonies of *A. mellifera* are severely compromised (ALPATOV 1977). Within the last twenty years *V. jacobsoni* has extended its range from Asia to Eastern and Western Europe. Northern Africa and much of South America (CRANE 1978, 1979).

The mites cause damage to adult and developing honey bees as they feed on the bee's hemolymph. In colonies with low populations, the developing bees may lose 5 to 25 % of their weight; occasionally there is damage to wings or other appendages. However, the signs are easily missed as only a small percentage of emerging bees are obviously deformed even when over 50 % of the brood cells are infested (DE JONG *et*

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al., submitted for publication). Most of the damaged bees are immediately removed from the colony by house-cleaning bees (DE JONG, unpublished data). When infestations have increased to the point that they are noticeable, the mite populations are already quite high (DE JONG and GONÇALVES 1981). During the three to six years necessary for a new infestation to reach this point, the mites are widely dispersed by drifting bees, swarms and the movement of colonies by man (PHADKE *et al.*, 1966, SMIRNOV 1978). It is therefore desirable to locate new foci of infestation early so that the mites can be controlled or eliminated.

There are two principal ways of examining bee colonies for the presence of *V. jacobsoni*. One method is to uncap brood cells and remove the larvae or pupae for examination. Since the mites have a preference for drone brood (RITTER and RUTTNER 1980), examining drone cells is usually more productive. A second method involves an examination of the adult bees by shaking them in a solution which will dislodge the mites. Large numbers of bees can be sampled in a short time by this method, facilitating the detection of low-level infestations and providing a means for estimation of mite populations.

Varroa jacobsoni that are attached superficially to the bee are easy to remove by shaking bees in a liquid phase. However, mites that are attached to the intersegmental membranes of the abdominal sternites or tergites are difficult to see and to remove even if the bees are dead. Several types of solutions have been used for shaking bees to detect *V. jacobsoni*. These include hot water (GROBOV 1977), detergent solutions (STOLBOV and VASIKOV 1976), hexane or gasoline (RITTER and RUTTNER 1980) and 96 % ethanol (DE JONG and GONÇALVES 1981). Gasoline is reportedly nearly 100 % effective for separating mites from adult bees (RITTER and RUTTNER 1980); however, it is dangerous to use as the fumes are toxic and highly flammable. In Brazil ethanol was found equally effective (GONÇALVES *et al.*, 1980), but in most other countries it may be costly and not readily available.

Since the shaking technique is used frequently for the detection of *V. jacobsoni*, we decided to test the various products to compare their efficiency for extracting mites. Solutions were selected according to availability and facility of use. We tested each solution with and without the use of a laboratory shaker. Dried samples were also tested to determine the suitability of winter-killed bees or dried samples sent to a central laboratory for diagnosis.

MATERIALS AND METHOD

A plastic shaking container developed in Brazil (DE JONG and GONÇALVES 1981) was used for all of the experiments. It consisted of a 1-l plastic alcohol bottle that had a constriction in the middle. The middle third section of the construction was cut out and the portions reassembled. A round piece of wire screening with openings 3 mm square (these should be larger than 1.9, but smaller than 4.5 mm) was cut slightly larger than the inside diameter of the bottle, then the screen was fitted into the upper end of the

bottle from the inside. With the bottle cap in place the inverted bottle assembly retained the bees above the screen while the mites dropped down to the neck of the bottle (Fig. 1). Where such a container is not

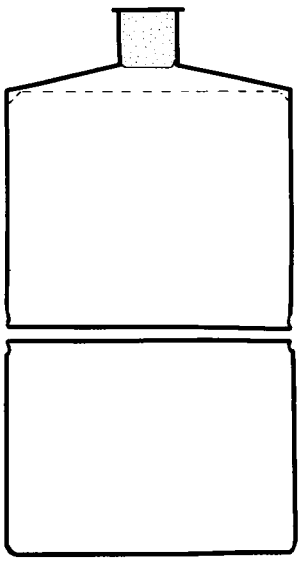

FIG. 1. — *Diagramatic sketch of container for shaking bees.* The bottom (shown here separated from the top) contains a screen (dashed line) through which the mites fall.

available, a shaking container can be made by cutting the bottom off any plastic bottle and using a piece of flexible plastic wrap secured with a piece of elastic as a cover for the bottom. Several small holes about 0.5 mm diameter should be made in the top of the shaker or in the plastic wrap to allow for the release of air pressure caused by the evaporation of fluids during the shaking process. Adult bee samples were collected by brushing them from brood combs into a beaker or other wide-mouth container with 150 ml of shaking solution. The bees and solution were then poured into the shaking container (with cap in place) and enough extra solution added to cover all of the bees. Each bottle was agitated by hand with the cap down and moved in a circular motion for one minute. The solution was then poured into a bowl lined with a white small mesh cloth. The cloth was then lifted from the bowl and the mites on the cloth counted. The bottle was then recapped and refilled with the same solution and subjected to 30 minutes of circular agitation in a laboratory shaker. Additional mites separated by the second shaking were counted as before. As a final check all of the bees were examined individually for remaining mites.

In a separate test, two samples of live bees were placed into empty jars and killed by freezing overnight. One sample was dried in the sun for 24-hr. at 24-27 °C and the other oven-dried at 60 °C for 2-hr. The bees were then subjected to the shaking procedure using 96 % ethanol.

RESULTS AND DISCUSSION

The mean number of mites per samples was .156 per bee, with a range of .0237 to 583 (Table 1). Hand-shaking for one minute was reasonably effective, but for all solutions tested, mechanical shaking for 30 minutes effectively removed 100 % of the mites. Only rarely were mites encountered on bees after they had been through the mechanical shaking.

Several of the solutions were inconvenient to use. Many of the bees in water 25 °C (77 °F) were able to crawl out of the container. This was less of a problem with water at 40 °C (104 °F). The hot water, 60 °C (140 °F), and 100 °C (212 °F) was difficult to handle without an insulated container. Bees quickly succumbed in the

TABL. 1. — *Number of mites detected with shaking solutions.*

Solution	No. mites			No. bees
	Hand-shaking one minute	Mechanical shaker 30 minutes	Mites remaining on bees	
Ethanol				
15 %*	18	5	0	317
25 %	32	2	0	246
25 %	56	4	0	279
25 %	27	1	0	410
50 %	153	2	1	368
70 %	62	5	0	413
96 %	26	1	0	224
96 %	47	0	0	278
96 %	131	5	0	277
Isopropyl				
25 %	43	2	0	258
25 %	15	4	0	295
50 %	38	4	1	294
50 %	33	1	0	391
*Water**				
25 °C	11	6	1	202
40 °C	5	2	0	295
40 °C	8	14	0	411
40 °C	66	13	0	203
60 °C	11	2	0	267
100 °C	10	2	0	420
100 °C	13	4	0	389
*Tween 80**				
1 %	27	6	0	407
ADECID-C				
1 %	104	4	0	281
ODD				
1 %	20	0	0	357
1 %	142	6	0	254
SDS				
.4 %	23	0	0	430
.4 %	21	3	0	458
.4 %	97	6	0	230

* Percentage of total mites detected by hand-shaking alone significantly less (P .05) than that obtained with 96 % ethanol (contingency table analysis with correction for continuity, Snedecor and Cochran, 1980). Data were lumped for each kind of solution, except for ethanol solutions which were separated according to concentration.

Aqueous detergent solutions.

Tween 80, Merck.

ADECID-C 40 % Polyethlene-nonyl phenyl ether, 60 % inert ingredients. Mitsui Ihara, S.A.

ODD Orinex S/A, São Paulo, Brazil. This is a commonly used liquid dishwashing detergent manufactured and distributed in Brazil; formula not revealed.

SDS Sodium dodecyl sulfate. Electrophoresis purity reagent. Bio-Rad laboratories. Richmond, California.

detergent solutions as well as in the ethanol and isopropyl alcohol. The foam in the detergent solutions sometimes made it difficult to count mites, though rinsing the filter cloth with additional water usually eliminated this problem.

The results from the various solutions were compared to those obtained with 96 % ethanol to determine if they were equally effective in extracting the mites using only hand-shaking. Water, Tween-80, and 15 % ethanol were significantly less efficient. However, there was no significant advantage to using 96 % ethanol over the remaining alcohol solutions.

The shaking procedure using dried bee samples proved unreliable. Over 10 % of the mites remained attached to the dried bees even after 30 minutes of mechanical shaking in 96 % ethanol (Table 2). In contrast, 100 % of the mites were dislodged from freshly killed bees by the same treatment.

TABL. 2. — *Number of mites detected in air dried bee samples shaken in 96 % ethanol.*

	found in container after freezing	fell off during drying	hand shaking one minute	mechanical shaker thirty minutes	remaining mites
(868) bees oven dried at 60 °C for two hours	2	4	10	9	5
(848) bees sun-dried for three hours then 24 hours at 24-27 °C	2	8	5	3	7

CONCLUSIONS

Shaking bees in various solutions is an efficient means of detecting the presence of *V. jacobsoni* in samples of freshly killed adult honey bees. Twenty-five percent solutions of ethanol or isopropyl alcohol were effective, convenient to use, and reasonable in cost. Hand-shaking for one minute removed 79-96 % (average 92 %) of the mites when 25 % alcoholic solutions were used. Mechanical shaking on a rotary shaker for 30 minutes using 25 % alcoholic solutions removed 100 % of the mites. The difference in efficiency of mite removal between hand-shaking and mechanical shaking was statistically significant.

Dried adult bee samples, which are more easily shipped to a central laboratory, can be diagnosed with a shaking solution, although the method is not as reliable as when freshly killed bees are used.

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RÉSUMÉ

ÉTUDE COMPARATIVE DE LA DÉTECTION DE *VARROA JACOBSONI* PAR AGITATION DES ABEILLES DANS DES SOLUTIONS

Secouer des abeilles dans diverses solutions est un moyen efficace pour détecter la présence de *Varroa jacobsoni* dans des échantillons d'abeilles adultes fraîchement tuées. Des solutions d'éthanol ou d'alcool isopropylique à 25 % sont efficaces, faciles d'utilisation et d'un coût raisonnable. L'agitation manuelle dans des solutions alcooliques à 25 % pendant une minute a éliminé 79-96 % (en moyenne 92 %) des acariens. L'agitation mécanique sur un agitateur centrifuge pendant 30 minutes dans des solutions alcooliques à 25 % a éliminé la totalité (100 %) des acariens. La nature de la solution, qu'il s'agisse de solutions alcooliques, de détergents ou d'eau chaude, ne semble pas être décisive dans le cas de l'agitation mécanique. La différence d'efficacité entre l'agitation manuelle et l'agitation mécanique est statistiquement significative.

On peut établir un diagnostic sur des échantillons d'abeilles adultes séchées, plus faciles à expédier à un laboratoire central, par agitation dans une solution, bien que la méthode ne soit pas aussi fiable qu'avec des abeilles fraîchement tuées.

Le récipient utilisé pour le test d'agitation est une bouteille d'alcool en plastique d'un litre, possédant un rétrécissement dans son milieu. Le tiers médiant de la bouteille avec le rétrécissement a été supprimé et les deux portions rassemblées après avoir ajusté de l'intérieur un morceau circulaire de grillage métallique, ayant une maille de 3 mm² (> 1,9 et < 4,5 mm), dans la partie supérieure de la bouteille.

ZUSAMMENFASSUNG

VERGLEICHENDE PRÜFUNG VON SCHÜTTELLÖSUNGEN FÜR DAS AUFFINDEN VON *VARROA JACOBSONI* AN ADULTEN BIENEN

Das Schütteln der Bienen in verschiedenen Lösungen ist ein wirkungsvolles Mittel, um das Vorhandensein von *Varroa jacobsoni* in Proben von frisch getöteten adulten Honigbienen zu entdecken. 25 % ige Lösungen von Äthanol oder Isopropylalkohol zeigten ein gutes Ergebnis, waren angenehm im Gebrauch und günstig in den Kosten. Schütteln von Hand durch 1 Minute entfernte 79-96 % (im Mittel 92 %) der Milben, wenn 25 % ige Alkohollösungen benutzt wurden. Mechanisches Schütteln für 30 Minuten in einem rotierenden Schüttler entfernten in einer 25 % igen Alkohollösung 100 % der Milben. Die Art der Schüttellösung schien nicht entscheidend zu sein – gleichgültig, ob es sich um alkoholische Lösungen, um Detergentien oder um heisses Wasser handelte, sofern ein mechanischer Schüttler benutzt wurde. Der Unterschied im Wirkungsgrad der Milbenentfernung zwischen Schütteln von Hand oder mechanischem Schütteln war statistisch signifikant.

Auch Proben von getrockneten adulten Bienen, deren Versand zu einem Zentrallaboratorium einfacher ist, können mit einer Schüttellösung diagnostiziert werden, aber die Methode ist nicht so verlässlich wie bei Verwendung von frisch getöteten Bienen.

Als Schüttelgefäss wurde in diesem Versuch eine 1-Liter Plastik-Alkoholflasche mit einer Einnehmung in der Mitte benutzt. Das mittlere Drittel der Flasche mit der Einnehmung wurde herausgeschnitten, die Teile wurden nachher wieder zusammengesetzt. Ein rundes Stück Drahtgitter mit Maschen von 3 mm² (> 1,9 < 4,5 mm) wurde von innen in das obere Ende der Flasche eingepasst (Abb. 1).

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