Re-examination of the Accuracy of a Detergent Solution for Varroa Mite Detection

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Abstract

Three methods for detecting varroa mites on adult honey bees were re-examined using large sample sizes: 1) hand-stirred in 70% ethanol for one minute, 2) hand-stirred in detergent solution for one minute, and 3) mechanical agitation using detergent solution for 30 minutes. Our results showed that the use of dishwashing detergent is an effective and economical alternative to alcohol for mite detection on adult bees. We conclude that shaking the bees with a mechanical shaker makes the method more accurate.

Introduction

The ability to accurately and easily evaluate colonies for varroa mite infestation is a critical part of successful beekeeping. However, the time and cost of detection is a concern not only for beekeepers, but bee researchers as well. Many techniques have been developed to detect or measure varroa populations in colonies. Examination of brood and emerging bees may be the most sensitive methods, but these procedures are labor intensive and can be done only during the brood rearing period (Webster and Callaway 1992). The use of acaricidal smoke such as fluvalinate and amitraz is more rapid, but contaminates honey and may accelerate the development of resistance to these chemicals by the mites (Ellis et al.1988, Herbert et al. 1989, Witherell and Bruce 1990). Varroa on adult bees can be detected using powdered sugar and other dusts in the laboratory or in the field (Fakhimzadeh 2001, Macedo et al. 2002). With this technique, bees can be returned to the colony and there is no risk of bee product contamination. The use of sticky board traps for varroa mite detection was also studied by several researchers (Calderone 1999, Sammataro et al. 2002, Parkman et al. 2002). This method does not require disruption of the colony by removing frames while sampling the entire adult bee population. However, this technique has the disadvantage of being time-consuming, since it takes two trips to the beeyard to obtain the results. Varroa on adult bees can also be detected using the ether roll technique. This method is rapid since it is done on location and materials are readily available (Ellis et al. 1988). However, not all varroa mites stick to the sides of the jar. Varroa mites conceal themselves under sternites to conceal themselves from the grooming activities of adult bees, which may protect them from being dislodged during the shaking process. Thus, washing of adult bees is probably the best way to dislodge varroa mites.

Collecting worker bees and storing them frozen for later processing by washing mites from the stored bees is a common method. Many washing solutions have been tried but many are expensive and also pose health problems. The use of dishwashing detergent solutions and ethanol are the most common solutions being used. Agitation or shaking improves the sensitivity of the methods. De Jong et al. (1982) compared the use of different shaking solutions. Unfortunately, they examined only a few samples. Using larger samples of bees collected and frozen for later analysis, our study re-evaluated the effectiveness of washing adult bees in alcohol and detergent solutions by hand-stirring and using a mechanical shaker.

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Materials and Methods

Seventy-one traps containing pheromone lures were placed in three locations around Baton Rouge, Louisiana in April 2003. After 2 months, the 42 surviving swarms from the traps that had brood were processed. From each trap, all combs were removed and as many bees as possible were brushed into a plastic bag. These collections of adult bees were frozen and stored for further processing.

Each colony sample was divided into three sub-samples, each of which was assigned to one of three initial washing methods: a) bees were hand-stirred in 70% ethanol for one minute, b) bees were hand-stirred in detergent solution for one minute, and c) bees were agitated using a mechanical shaker in detergent solution for 30 minutes (details below). Since sub-samples were large (208-1381 bees), each sub-sample was then divided into 1 to 4 groups of bees depending on the size of the sub-samples. A total of 392 groups with an average count of 591 ± 8 bees were used to evaluate the three washing methods. In order to determine the accuracy of the three methods, groups of bees were washed three times using three orders of the three washing methods (a>b>c, b>c>a, and c>a>b). For example, after washing a group of bees in alcohol, it was then washed with detergent solution by hand and finally by a mechanical shaker to remove all the remaining mites.

To separate bees from varroa mites, plastic trays and large-mesh screen baskets (Harbo 1988) were used for methods 1 and 2. Adult bees were placed in the basket and stirred by gloved fingers in ethanol or detergent solution for one minute. Before taking out the screen basket, the basket was plunged in and out of the washing solution to remove any lodged mites. The time spent from stirring to the end of the bee and mite counts was recorded.

The container used to agitate samples was assembled as follows: the bottom third of a 1 quart plastic container was removed and replaced with a 1/8 inch mesh hardware cloth glued in its place. The bees were put in the screened container, which was then fitted into

an unaltered 1 quart plastic container to hold the water. The nested containers were then filled with enough cold water, 1-1.2 pints (500-600 ml), to cover the bees. About one teaspoon (1 to 2 ml) of dishwashing detergent (Sun Light®) was added. All containers had lids to prevent the contents from spilling. A mechanical, reciprocating (back and forth) shaker (Eberbach, 1 inch (2.5 cm) travel, 150 cycles/min) fitted with a wooden tray having compartments to hold 20 containers was used. After 30 minutes, the screened container with the bees was separated from the container holding the detergent solution. The bees remained in the screened container and the mites quickly sank to the bottom of the container holding the detergent solution. The numbers of both bees and mites were counted. The time spent from turning on the shaker to the end of the bee and mite counts was recorded. The average shaking time per group was calculated when more than one group was processed at the same time. The total number of mites for each sub-sample was determined by adding the numbers of recovered mites in the first, second and third washes. The number of mites recovered from each wash was then divided by the total number of mites and multiplied by 100 to determine the accuracy (%) of each method.

Data were analyzed using ANOVA in a completely randomized design using mixed procedure. Before analyses, all data were transformed using square root transformation (SAS Institute 1997). The accuracy of only the first wash for each method was compared.

Results and Discussion

A quality method to detect and quantify varroa infestation in honey bee colonies is an important part of successful beekeeping. Sampling of adult bees is quick and can be done in any season, regardless of the status of a colony's brood nest. When mite populations in individual colonies are evaluated, it is typical that only one sample is taken and evaluated at one time using one method. Since this study compared methods of removing mites from samples of bees, we took advantage of the large number of adult bees (5,530 ± 317, range 724-9091) found in the captured swarms. Each swarm provided three sub-samples that could be initially washed using one of the three methods being evaluated. All of these swarms were infested by relatively low numbers (1.16 mites per 100 adult bees) of varroa mites (1.16 ± 0.17%, range 0.08-5.84%).

Our results indicate that the use of detergent solution with mechanical agitation as a single wash was the most effective (97%) way of detecting varroa mites (P= 0.004) (Table). The accuracies (P= 0.3349) of the two hand-stirred methods (alcohol and detergent solution) were comparable.

Table. Comparative accuracy of three methods for varroa detection on adult bees.

Treatment	Accuracy (first wash)*	Second wash*	Third wash**	Average time per 100 bees + mites (min)*
Hand-stir in alcohol	89±2%^{b}	10±1%^{a} (Hand/deterg)	0.9±0.6% (Mech/ deterg)	1.21±0.06^{b}
Hand-stir in detergent solution	91±2%^{b}	6±1%^{a} (Mech/deterg)	3±0.6% (Hand/ alcohol)	1.25±0.06^{b}
Mechanical shaker + detergent solution	97±2%^{a}	2±1%^{b} (Hand/ alcohol)	0.2±0.6% (Hand/ deterg)	2.79±0.06^{a}

* Means followed by the same letter are not significantly different (P=0.05)

** not significant

Method inside ( ) indicates the method used for the second and third washes.

Time and cost of detection are very important criteria when considering a method to use. The agitation with detergent method significantly (P= 0.0001) took the most time, while the two hand-stirred methods (alcohol and detergent solution) equally took less time (P= 0.4328). However, most of the time involved with the shaker method was machine time. Also, since the shaker device can hold up to 20 containers at one time, the actual time per sample spent using this technique is significantly lower.

There are several factors that may have limited the full (100%) accuracy of the two detergent methods. Mites in the detergent foam may easily be missed (De Jong et al. 1982). Rinsing the bees with running water with a container to catch any mites may help achieve full accuracy. Also, lids from the containers can be rinsed to remove mites that may have become lodged near the closure ring. A very large number of bees per container may restrict the removal of mites. In this study, the number of bees per container ranged from 208 to 1381 bees (average = 591 ± 8 bees). If each container of this size had 300-400 bees, it would assure that the bees were not packed tightly and would allow mites to be dislodged easily. Larger containers could reasonably be expected to be more effective with larger numbers of bees. Another possible factor was the condition of the bees. Most of the samples were sticky; honey dripped as the whole colonies were harvested. The presence of honey may have made it more difficult to wash mites from the bees. When samples are taken from hives, the bees are only sticky during very strong nectar flows.

Harbo (1988) suggested that ethanol should be used for washing bees since varroa tends to float in water. The addition of detergent to water eliminates this difficulty: mites quickly sank to the bottom of the containers with a detergent solution after agitation of the solution was stopped.

Powdered sugar is useful for immediate tests in the apiary of smaller numbers of samples, particularly where the beekeeper desires to return the bees unharmed to the colony (Macedo et al. 2002). However, the powdered sugar assay would usually be done by a trained beekeeper. Washing bees is more suitable where large numbers of samples must be processed, so that in the apiary processing is not an optimal use of beekeeper time. Samples can be processed at a central facility by workers who do not need to be trained beekeepers.

Since alcohol is more expensive and its vapor is unpleasant and may pose health hazards, we conclude that the use of water with a small amount of dishwashing detergent is an effective and economical alternative for detecting varroa. Alcohol may also be more difficult to obtain, is more hazardous to store and use, and requires more careful handling for safe and proper disposal. We confirmed that shaking the bees with a mechanical shaker makes the method both more rapid and more sensitive.

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