



A simplified technique for counting *Varroa jacobsoni* Oud. on sticky boards

Nancy Ostiguy, Diana Sammataro

► To cite this version:

Nancy Ostiguy, Diana Sammataro. A simplified technique for counting *Varroa jacobsoni* Oud. on sticky boards. *Apidologie*, 2000, 31 (6), pp.707-716. 10.1051/apido:2000155 . hal-00891743

HAL Id: hal-00891743

<https://hal.science/hal-00891743>

Submitted on 11 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Original article

A simplified technique for counting *Varroa jacobsoni* Oud. on sticky boards

Nancy OSTIGUY*, Diana SAMMATARO

Department of Entomology, Pennsylvania State University, University Park, PA 16802, USA

(Received 21 July 1999; revised 11 January 2000; accepted 25 August 2000)

Abstract – The most common method used to assess the level of mite infestation in a bee colony is to count all the mites that fall onto sticky boards placed on the bottom of a colony. Unfortunately, this is a laborious and boring task. Therefore, a stratified sampling technique was devised to accurately estimate the number of mites on sticky boards. The technique, when compared to a census count of all mites, resulted in a coefficient of determination of 0.97 or greater. The stratified sampling protocol in which we randomly selected 33% of the cells on a sticky board and did not choose new random numbers for each sticky board resulted in an accurate estimate of the number of *Varroa jacobsoni*. This technique gave a mean percent error of $0.4\% \pm 9.5\%$ for any one estimate of a sticky board.

stratified sampling / *Varroa jacobsoni* / mite / sticky board

1. INTRODUCTION

Varroa jacobsoni live exclusively on honey bees and have become a serious pest worldwide. It has become important for both beekeepers and researchers to regularly monitor levels of mites in honey bee colonies. The most common method for assessing the level of mite infestation that is not destructive to bees entails counting adult mites that fall to the bottom of the colony and are captured on a board placed on the

bottom of the hive [2–7]. These boards are called sticky boards when they are coated with a sticky substance, such as petroleum jelly to ensure that the mites that fall onto the board remain until each has been counted. Each mite on the sticky board is counted and the total number of mites is divided by the number of days the board was in the hive to determine the mite drop per day.

Counting each mite on a sticky board is a laborious and boring task. In highly infested colonies, there may be thousands of mites to

* Correspondence and reprints
E-mail: nxo3@psu.edu

count. The mites are tiny, and hidden among other debris that falls onto the sticky boards. As a result, we find that there is considerable inconsistency in making a complete census of the number of mites on a board.

Rather than taking a complete census, a sampling technique was developed to estimate the mite counts. If mites were evenly distributed on sticky boards, counting the number of mites in a simple random sample of cells would be an efficient and accurate method for determining mite numbers. Two factors create a non-random distribution of mites. Mites are not evenly distributed on a board because of the physical nature of

the beehive. Inside a hive, bees live on series of parallel frames of comb. The distance between frames causes mites to land on the sticky board in bands. The width of the bands is determined by the distance between the frames and the frame width. In addition, bees are not distributed uniformly on the frames, but usually cluster in the center of the hive. As a result, mites tend to occur on the sticky boards non-evenly in parallel bands (Fig. 1).

A simple random sample of non-random data can often over or under sample different portions of a population. A method for estimating the number of mites on sticky boards using stratified random sampling was developed by Calderone [1]. His method did not respect the 2 dimensional stratification produced by hive characteristics and mite/bee behavior. We developed a stratified sample technique to take full advantage of the striped and clumped pattern of *Varroa jacobsoni* on sticky boards. Stratification divides the population into groups based upon the natural distribution of the data within the population. Samples are then taken from within each group to ensure that the sample represents the actual population. In addition, we used new statistics not used by Calderone to evaluate our sampling algorithm. This stratified sampling technique was evaluated for its ability to accurately estimate the total number of mites on a sticky board. In addition, we compared the accuracy of the counts when different proportions of the sticky board were counted. Finally, we assessed the need to select new random numbers for each sticky board versus using the same random numbers for every sticky board.

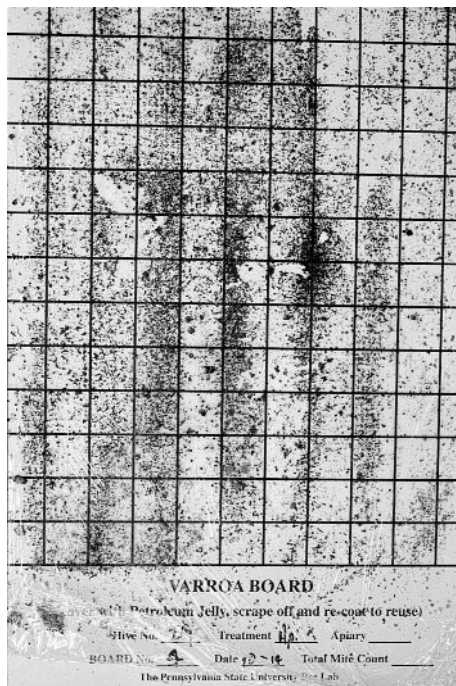


Figure 1. Non-random mite distribution on a sticky board. Mites are not evenly distributed on the sticky board because of the physical nature of the beehive and the behavior of bees. The frames cause mites to land on the sticky board in parallel strips. Bees cluster within the hive. The result of these two factors is that mites tend to occur within non-evenly distributed parallel bands.

2. MATERIALS AND METHODS

2.1. Sticky boards

For our sticky boards, we use sheets of waxed cardboard, coated with petroleum jelly, to trap fallen mites. The dimensions

of the sticky board are 32 cm by 42 cm. A grid containing two hundred and sixteen cells (12 cells $\times$ 18 cells) is printed on the sheets. Each cell is two square centimeters. The cell size was chosen to match the distance between hive frames, and the size of the grid (24 cm by 36 cm) corresponds with the area within the hive where mites fall.

2.2. Counting techniques

We used several techniques to count mites. First, we did complete counts of the total number of mites on fifty-four sticky boards. This census was assumed to be the true and accurate count of mite numbers. The mite fall pattern on these fifty-four boards was used to design the sampling procedure. To take advantage of the mite fall pattern in the sampling of the sticky boards, the 216 two by two centimeter cells on each grid were grouped into blocks of nine cells

(Fig. 2). Each cell within a block was assigned a number from one to nine; a random number generator was used to produce a list of numbers. Cells were selected randomly without replacement from each block using a random number table [8]. Some mites fall on the lines of the grid rather than within a cell. To ensure an accurate count, mites on a grid line were included in a cell count if they fell on the left or upper lines of the cell. A mite on the right or lower cell line was considered to belong to the adjacent cell. Because the same number of cells are sampled in each strata it is possible to determine the total number of mites on a sticky board by multiplying the mean number of mites per cell by the total number (216) of cells.

We evaluated six different sampling procedures based upon two variables. The first variable was the percentage of the board counted. Either two, three or four cells

1	2	3	1	2	3	1	2	3	1	2	3
4	5	6	4	5	6	4	5	6	4	5	6
7	8	9	7	8	9	7	8	9	7	8	9
1	2	3	1	2	3	1	2	3	1	2	3
4	5	6	4	5	6	4	5	6	4	5	6
7	8	9	7	8	9	7	8	9	7	8	9
1	2	3	1	2	3	1	2	3	1	2	3
4	5	6	4	5	6	4	5	6	4	5	6
7	8	9	7	8	9	7	8	9	7	8	9
1	2	3	1	2	3	1	2	3	1	2	3
4	5	6	4	5	6	4	5	6	4	5	6
7	8	9	7	8	9	7	8	9	7	8	9
1	2	3	1	2	3	1	2	3	1	2	3
4	5	6	4	5	6	4	5	6	4	5	6
7	8	9	7	8	9	7	8	9	7	8	9
1	2	3	1	2	3	1	2	3	1	2	3
4	5	6	4	5	6	4	5	6	4	5	6
7	8	9	7	8	9	7	8	9	7	8	9

Figure 2. Sampling grid: two, three or four cells were randomly selected from each of the 24 blocks on a sticky board to estimate the number of mites. The shading of the 3 cells within each block provide an example of the template developed to save time by pre selecting the randomly chosen cells from which mites are counted.

within each block of nine cells were counted, resulting in forty-eight cells (22%), seventy-two cells (33%) or ninety-six cells (44%) cells, respectively, being counted. The mean number of mites per cell was determined for each of the three sample sizes and used to estimate the total number of mites per board. Then, for each of the three sample sizes we used two methods of cell selection. In one case, new sets of randomly selected cells were counted for each board. This was more time consuming than simply using the same set of randomly selected cells for all the boards. Therefore, we compared the results of using a new set of random cells for each board with that of using a single set of randomly selected cells for all the boards.

2.3. Statistical analysis

The accuracy to which the estimated mite number predicts the census count of mites was evaluated using simple linear regression. Regression equations were compared for the six sampling procedures. We also compared the accuracy of the six sampling procedures in cases where there were low, medium or high numbers of mites on the boards (< 500, 500–1 000, or > 1 000 mites per board).

Two methods were used to estimate the degree of error expected from the sampling procedures. The first method, termed the overall mean error, is the error associated with estimating mite number on many sticky boards. The error estimates for each board are combined to provide a mean error estimate for the population of boards counted rather than an error estimate for a single board. The second method described, the mean percent error, is the error associated with estimating mite numbers on one sticky board. This error estimate provides an expected error estimate for one board rather than the population of boards.

The degree to which the estimated mite count for a single sticky board could be in

error can be determined by taking the absolute value of the census mite count minus the estimated mite count and then dividing by the census mite count. If the error for each sticky board is summed, then you have a cumulative error estimate for all the sticky boards combined. The cumulative error estimate is called the overall mean error. The overall mean error was determined by the following formula:

Overall mean error

$$= \sum_{i=1}^n \frac{|count_i - estimate_i|}{count_i}$$

where the *count* is the census count of mites on the sticky board, the *estimate* is the number of mites estimated for each procedure, *i* is the sticky board number and *n* is the sample size. Because we are summing the absolute value of the census count minus the estimated count for each sticky board, this measure cannot have any negative values and will never have a sum less than zero. If the estimation procedure is accurate, the overall mean error and its standard deviation should be small. Overall mean error and its standard deviation were calculated for each sampling proportion, 22%, 33%, and 44%, for each mite density, low, medium, high and overall, and for new versus the same randomly selected cells.

To evaluate the error in predicting specific values for each sticky board, the mean percent error and its standard deviation were calculated for each sampling proportion, for new versus same randomly selected cells and for each mite densities. Unlike the overall mean error, the mean percent error yields both positive and negative values and can have an overall value close to zero even if the ability to accurately predict a particular value is low. Thus standard deviation provides a more useful measure of the probability of obtaining an estimated value near the census count value. The smaller the standard deviation of the mean percent error, the more confident you can be that a

particular estimate is close to the actual census count. The formula used was:

Mean percent error

$$= \sum_{i=1}^n \frac{\text{count}_i - \text{estimate}_i}{\text{count}_i},$$

where *count* is the census count of mites on the sticky board, *estimate* is the number of mites estimated for each procedure, *i* is the sticky board number and *n* is the sample size.

Concordance correlation [8] is an additional statistical method used to compare the reproducibility of measurements. This technique can be used to compare a new measurement procedure against an accepted procedure. A high, positive correlation indicates no difference between the new and the old procedures. Using concordance correlation, we compared the stratified random sampling techniques used to estimate the number of mites with the census counts.

3. RESULTS

Initially, we compared the sampling estimates using a single set of randomly selected cells for all the boards with estimates using a new set of cells for each board. No significant difference in predictive ability

was observed between methods regardless of the percentage of the board counted (22%, 33% or 44%) or the numbers of mites per board (< 500, 500–1 000, > 1 000, and all boards). Consequently, only the results for the single set of randomly selected cells are presented.

For each sample size used to estimate mite numbers, the ability of the estimated number of mites to predict the census count was determined. The estimated number of *Varroa jacobsoni* was able to explain no less than 94% of the variation in the mite census count (Tab. I). For sticky boards with more than 500 mites, the predictive value of the estimated mites number did not improve when more than 33% of the sticky board was counted. For sticky boards with less than 500 mites, the greatest improvement in the predictive ability of the estimated mite number occurred when 33% of the sticky board was counted.

The overall mean error was the lowest when 44% of the sticky board was counted and over 1 000 mites were present while the largest overall mean error occurred where 22% of the board was counted and fewer than 500 mites were present (Tab. II). A substantial drop in the overall mean error was seen when at least one-third of the cells was counted.

Table I. Coefficient of determination for different density sticky boards and the percent of board counted. The proportion of the sticky board counted and the density of mites influence the ability of the estimated number of mites to predict the census count of mites. For each proportion and mites density of the coefficient of determination (R^2) is given. Ninety nine percent of the census count value can be explained by the estimated value when at least 33% of the board is counted and the board has a mite density greater than 500.

	Percent of sticky board counted		
	22%	33%	44%
All boards	0.99*	0.99*	0.99*
Boards with < 500 mites	0.94*	0.97*	0.98*
Boards with 500–1 000 mites	0.98*	0.99*	0.99*
Boards with > 1 000 mites	0.98*	0.99*	0.99*

* $P = 0.0001$.

Table II. The overall mean error for different density sticky boards and the percent of board counted. The overall mean error, which is the summation of the absolute value of (census minus the estimated mite number) divided by the census number for each sticky board, is a cumulative error estimate for all the sticky boards. This measure will never be less than zero. If the sampling procedure is accurate, the overall mean error and its standard deviation should be small.

	Percent of sticky board counted		
	22%	33%	44%
All boards	8.4% $\pm$ 7.2%*	6.5% $\pm$ 6.5%	6.1% $\pm$ 5.6%
Boards with < 500 mites	9.4% $\pm$ 8.8%	8.3% $\pm$ 8.1%	7.5% $\pm$ 6.8%
Boards with 500–1 000 mites	8.3% $\pm$ 5.7%	4.6% $\pm$ 3.7%	5.5% $\pm$ 4.1%
Boards with > 1 000 mites	6.7% $\pm$ 4.5%	5.0% $\pm$ 4.2%	4.2% $\pm$ 3.3%

* Overall mean error $\pm$ standard deviation.

With this sampling technique, there is a tendency to underestimate the number of mites. The mean percent error for any estimate was not greater than 1.6% and the largest standard deviation was 12.9% (Tab. III). When fewer than 500 mites were on a board, for any estimate of a sticky board the probability was 68 percent that the error would be within 12.9% of the census count; this was the worst estimate of mite number per board. The best mite estimate was produced when there were more than 1 000 mites on a board and 44% of the board was counted; the probability was 68 percent that

the error would be within 5.3% of the census count. The least accurate estimate of mite number occurred when mite number was less than 500.

The concordance correlation was determined between the census counts of mites on a sticky board and the estimated number of mites (33% of the sticky board counted). The coefficient of determination was 0.9989 ($P < 0.001$), indicating a high degree of similarity between values obtained by both procedures for each board. The 95% confidence interval for the concordance correlation is 0.9988 to 0.9991.

Table III. The mean percent error for different density sticky boards and the percent of board counted. The mean percent error and standard deviation were calculated for each sampling proportion and for each mite densities. The mean percent error yields both positive and negative values and can have an overall value close to zero even if the ability to accurately predict a particular value is low. Thus standard deviation is the more useful measure. The smaller the standard deviation of the mean percent error, the more confident you can be that a particular estimate is close to the actual census count.

	Percent of sticky board counted		
	22%	33%	44%
All boards	1.5% $\pm$ 11.2%*	0.4% $\pm$ 9.5%	0.5% $\pm$ 8.6%
Boards with < 500 mites	1.6% $\pm$ 12.9%	0.8% $\pm$ 11.6%	1.2% $\pm$ 10.2%
Boards with 500–1 000 mites	1.5% $\pm$ 9.7%	1.3% $\pm$ 5.4%	1.4% $\pm$ 5.3%
Boards with > 1 000 mites	0.6% $\pm$ 8.3%	0.9% $\pm$ 6.7%	1.1% $\pm$ 5.3%

* Mean percent error $\pm$ standard deviation.

4. DISCUSSION

Even though a complete census may seem to provide an accurate count of mites on a sticky board, this assumption is not likely to be valid due to systematic and random error. Systematic error will occur because boards with greater mite density will be more difficult to count than boards with lower mite density. Additional systematic error is added when debris is present on the boards or mites are piled on top of one another. The more debris, e.g., wax and bee parts, on a sticky board, the more difficult it is to distinguish between a mite and debris. Random error occurs when mites are missed or mites are thought to be present when they are not. Even though census counting of mites is unlikely to provide the true count, the census count is the considered the most accurate method for assessing the level of mite infestation. Therefore, various sampling protocols were compared to the census count of mites on sticky boards to determine if a sampling of mites could replace a census count with little loss in count accuracy.

We used several techniques to evaluate the accuracy of the described technique. A concordance correlation is used when it is desirable to know if measurements are the same from two different methodologies, instruments, or technicians. Two different measurement techniques – the census mite count to the mite count estimated by sampling – were compared in this study. The second method used to evaluate the described technique was the overall mean error. Like a standard error, the overall mean error measures the error for the population of boards. The last method used was the mean percent error. This measurement is analogous to the standard deviation. It provides an estimate of how far each sampled board is from the total number of mites on that same board. All three measures are useful; the concordance correlation provides a specific statistical test for comparing two methodologies. The overall mean error provides an

assessment the accuracy of sampling a population of sticky board as compared to counting each mite on a sticky board. This measurement answers the question: how accurate is the method in determining the number of mites on all sticky boards combined? The mean percent error provides a way to compare the estimated number of mites on a sticky board to the census count of mites on the same sticky board. This measurement answers the question: how accurate is the method in determining the number of mites on each sticky board?

When sampling protocols were compared to the census count, it was possible to obtain an adjusted R^2 no smaller than 0.97, an overall mean error no larger than 8.3% (SD = 8.1%) and a mean percent error no larger than 0.8% (SD = 11.6%) by counting only one-third of each sticky board. If the mite count exceeds 1000 per board, it is possible to count only twenty-two percent of the sticky board and still obtaining an adjusted R^2 of 0.98, an overall mean error of 6.7% (SD = 4.5%) and a mean percent error of 0.6% (SD = 8.3%). It is possible that sampling will result in a more accurate mite count than attempting to count each individual mite due to the occurrence of systematic and random errors when performing a census count.

Using a sampling technique that takes advantage of the striped and clumped pattern of *Varroa jacobsoni* on sticky boards is essential for obtaining accurate estimates of the number of mites. The size of each cell, two by two centimeters, and the blocking of cells were chosen to correspond to the observed mite pattern. If the stratified sampling technique does not match the mite pattern, the ability of the technique to estimate mite number will decrease precipitously, especially as the actual pattern deviates from the sampling pattern. Calderone [1] describes a stratified sampling technique that takes advantage of the parallel bands of mites formed by the combs but does not account for non-even distribution of mites caused by the clustering of bees. The Calderone

protocol provides a good estimate of mite number when the sticky board contains more than 500 mites and more than 50% of the board is counted. We used a sticky board with the same configuration as Calderone's. The improvement in the precision of the estimates produced by our method as compared to Calderone's can be explained by our full utilization of the stratified distribution of mites. Our sampling procedure improves on Calderone's work in three ways. The procedure: (1) is less complex; (2) needs only 33% of the board counted rather than more than 50%; and (3) is accurate for all mite densities rather than only densities exceeding 500 mites per board. Our results provide further evidence that the workload associated with counting mites can be greatly reduced by utilizing stratified random sampling.

A time-consuming aspect of sampling is the assignment of random numbers for each sticky board. We found that the selection of new random numbers for each sticky board does not increase the accuracy of the estimates. Therefore, a great deal of time can be saved by creating a template of randomly chosen cells. Based upon the results from this study, a new sticky board for estimating the number of mites has been developed. A grid with two hundred sixteen 2-cm cells (18 cells by 12 cells) has been printed onto a 32 cm by 42 cm board. Cells are grouped into blocks of nine cells with three cells per block randomly selected in each block and grayed (10% shade) to indicate the cells in which mites are to be counted. To estimate the mite drop per day, the number of mites in the shaded squares are counted. The total number of mites is then divided by 72 (the number of cells counted) and multiplied by 216 (the total number of cells on the board). Finally, the total number of mites is divided by the number of days the board was in the hive.

By using a stratified sampling protocol to randomly select 33% of the cells on a sticky board, it is possible to obtain an accurate estimate of the number of mites. This

sampling method will reduce the time necessary to evaluate the impact of various mite control techniques.

ACKNOWLEDGEMENTS

The work was supported in part by Pennsylvania Department of Agriculture grants ME447105 and ME446196, Fund for Rural America (USDA/CSREES 97-36200-5204), California State Beekeepers Association, The California Bee Breeders Association and the Mid-Atlantic Apiculture Research and Extension Consortium.

Résumé – Une technique simplifiée pour dénombrer *Varroa jacobsoni* Oud. sur les plateaux enduits de graisse. La méthode la plus répandue pour estimer l'infestation par l'acarien *Varroa jacobsoni* consiste à compter les acariens tombés et restés collés sur les plateaux de fond. Une technique d'échantillonnage stratifié a été développée pour exploiter la répartition en bandes et en agrégats de *V. jacobsoni* sur les plateaux. Si les acariens qui tombent se répartissent de façon uniforme, un échantillon simple de cellules prises au hasard constituerait une méthode efficace et précise pour estimer le nombre d'acariens. Mais comme les abeilles vivent par groupes sur des rayons parallèles, les acariens atterrissent sur les plateaux en bandes parallèles d'épaisseur variable (Fig. 1). Un carton enduit de cire et recouvert de vaseline a été utilisé pour piéger les acariens sur une grille de 24 sur 36 cm. Le plateau de fond comptait 216 cellules réparties dans une grille de 12 sur 18. La taille de la cellule, 2 cm², correspondait à la distance entre deux rayons de la ruche. Les cellules étaient groupées par blocs de neuf (Fig. 2) et choisies au hasard dans chaque bloc et sans remplacement. On a dénombré les acariens dans chaque cellule sélectionnée. Les acariens tombés sur une ligne de la grille étaient inclus dans le comptage d'une cellule s'ils étaient tombés sur le bord supérieur ou gauche de la cellule. On a évalué six procédures d'échantillonnage utilisant deux variables : (i) le pourcentage du plateau

soumis au comptage (22, 33 ou 44 %), (ii) la méthode de sélection des cellules (nouveaux groupes ou mêmes groupes de cellules choisies au hasard). Au total 54 plateaux ont été analysés. Les coefficients de détermination ne différaient pas quand on comparait les estimations utilisant le même groupe de cellules sélectionnées au hasard et les estimations utilisant un nouveau groupe de cellules. Le nombre d'acariens estimé pouvait expliquer au moins 94 % de la variation de la population d'acariens (Tab. I). L'erreur moyenne globale est associée au nombre d'acariens estimé pour la population de plateaux analysés ; elle a atteint sa valeur la plus faible lorsqu'on analysait 44 % du plateau et que plus de 1000 acariens étaient présents (Tab. II). L'erreur moyenne de pourcentage est associée au nombre d'acariens estimé pour 1 plateau et ne dépassait pas 1,6 % ; l'écart-type le plus élevé était de 12,9 % (Tab. III). Quand on analysait 33 % du plateau, la corrélation de concordance était de 0,9989 ($P < 0,001$), indiquant un fort degré de similitude entre les valeurs obtenues par les deux procédures. Bien qu'un recensement de tous les acariens puisse sembler fournir un comptage précis, cette affirmation n'est vraisemblablement pas valable en raison d'erreurs systématiques et aléatoires. Il existe une erreur systématique parce les comptages sur les plateaux ayant une grande densité d'acariens et de débris sont plus difficiles que sur les plateaux qui ont une plus faible densité d'acariens et moins de débris. L'erreur aléatoire survient lorsqu'on rate des acariens ou que l'on en compte à tort. Une technique d'échantillonnage qui prend en compte la répartition par bandes et par groupes des acariens tombés reste indispensable pour obtenir des estimations précises. Calderone [1] décrit une technique d'échantillonnage par couches qui prend en compte les bandes parallèles d'acariens mais pas la distribution non uniforme des acariens dans les bandes. Notre procédure d'échantillonnage améliore le travail de Calderone de trois façons : (i) elle est moins complexe, (ii) elle

ne nécessite de compter que 33 % du plateau au lieu de plus de 50 % et (iii) elle est précise quelle que soit la densité d'acariens, alors que celle de Calderone ne l'était que pour les densités supérieures à 500 acariens par plateau. En utilisant un protocole d'échantillonnage par couches pour sélectionner au hasard 33 % des cellules d'un plateau, il est possible d'obtenir une estimation précise du nombre d'acariens. Cette méthode réduira le temps nécessaire pour évaluer l'impact des diverses méthodes de lutte.

***Varroa jacobsoni* / échantillonnage stratifié / plateau enduit de graisse**

Zusammenfassung – Vereinfachte Schätzmethode zur Zählung von *Varroa jacobsoni* auf klebrigen Unterlagen. Bei der häufigsten Methode zur Bestimmung des Milbenbefalls müssen die auf der Unterlage klebenden Milben gezählt werden. Es wurde eine Methode mit Hilfe der stratified sampling Technik (Stichprobenwahl von sich nicht überlappenden Teileinheiten) entwickelt, um den Abfall von *Varroa jacobsoni* auf die Unterlagen in Streifenform bei gleichzeitigen Häufungen innerhalb der Streifen auszunutzen. Da sich Bienen im Bereich zwischen den Waben aufhalten, fallen die Milben in parallelen Streifen in unterschiedlicher Dichte auf die Unterlage (Abb. 1) Wäre der Milbenabfall gleichmäßig, würde eine einfache zufällige Stichprobe von Teileinheiten eine effiziente und zuverlässige Methode zur Schätzung der Milbenzahl sein.

Eine gewachste mit Vaseline überzogene Pappe wurde benutzt, um Milben auf einem 24 mal 36 cm Gitter aufzufangen. Die Unterlage enthielt 216 Flächeneinheiten in einem 12 mal 18 Gitter. Die Größe der Einheiten von 2 cm² entsprachen dem Abstand zwischen den Waben. Die Einheiten wurden in Neunerblocks aufgeteilt (Abb. 2) und ein Teil wurde zufällig und ohne Ersatz von jedem Block ausgewählt. In jeder ausgewählten Einheit wurden alle Milben gezählt.

Milben auf Gitterlinien wurden zu der Einheit gezählt, bei der sie auf der linken oder oberen Linie lagen. Sechs Stichproben wurden mit 2 Variablen ausgewertet: 1. wurden verschiedene Prozentsätze der Unterlage (22 %, 33 % oder 44 %) ausgezählt und 2. wurde die Methode der Auswahl von zwei Einheiten angewendet, wobei entweder ein neues oder ein gleiches Paar von zufällig ausgewählten Einheiten ausgezählt wurden. Insgesamt wurden 54 Unterlagen ausgewertet. Das Bestimmtheitsmaß (coefficient of determination) zeigte keinen Unterschied zwischen den Schätzungen mit zufällig ausgewählten Einheiten und denen eines neuen Satzes von Einheiten. Die geschätzte Anzahl der Milben konnte mindestens 94 % der Variation Milbenpopulation erklären (Tab. I). Der gesamte mittlere Fehler ist abhängig von der geschätzten Größe der Milbenpopulation, die auf der Unterlage gezählt wurde und war am niedrigsten, wenn 44 % der Unterlage ausgezählt wurde und es über 1 000 Milben gab (Tab. II). Der mittlere prozentuale Fehler ist abhängig von der Schätzung der Milbenzahl auf einer Unterlage und war nicht größer als 1,6 % und die höchste Standardabweichung betrug 12,9 % (Tab. III). Die Konkordanzkorrelation bei Zählung von 33 % der Unterlage betrug 0,9989 ($P < 0,001$), was einen hohen Grad von Ähnlichkeiten zwischen den Werten andeutet, die mit beiden Methoden gewonnen wurden. Obwohl eine Zählung aller Milben scheinbar die Bestimmung der genauen Milbenzahl ermöglicht, ist diese Annahme wahrscheinlich auf Grund systematischer und zufälliger Fehler nicht haltbar. Ein systematischer Fehler tritt auf, weil Unterlagen mit größerer Milbendichte und/oder Verschmutzung schwieriger zu zählen sind als Unterlagen mit weniger Milben und geringer Verschmutzung. Zufällige Fehler treten auf wenn Milben übersehen werden oder Milben angenommen werden, obwohl es sie nicht gibt. Ein Stichprobenwahl, die einen Vorteil aus den gestreiften und gehäuft Vorkommen von abgefallenen Milben zieht, ist wichtig, um genaue Schätzungen zu

erhalten. Calderone [1] beschreibt eine „stratified sampling technique“, die die parallele Streifung aber nicht die unregelmäßige Verteilung der Milben innerhalb der Streifen berücksichtigt. Unsere Stichprobenwahl verbessert die Methode von Calderone auf dreierlei Weise: 1. sie ist einfacher; 2. es müssen nur 33 % der Unterlage ausgezählt werden statt mehr als 50 % und 3. ist sie exakt bei allen Milbendichten und nicht nur für Dichten von mehr als 500 Milben pro Unterlage. Mit der „stratified sampling technique“ (zusammengesetzte Stichproben) von zufällig ausgewählten 33 % der Flächeneinheiten einer Unterlage ist es möglich, die Anzahl der Milben *Varroa jacobsoni* genau zu schätzen.

Diese Technik der Stichprobenwahl vermindert die Zeit, die man benötigt um die Wirkung verschiedener Behandlungsmethoden gegen die Milben abzuschätzen.

Zusammengesetzte Stichproben / *Varroa jacobsoni* / Milben / klebrige Unterlage

REFERENCES

- [1] Calderone N.W., Evaluating subsampling methods for estimating numbers of *Varroa jacobsoni* mites (Acari: Varroidae) collected on stickyboards, J. Econ. Entomol. 92 (1999) 1057–1061.
- [2] Chiesa F., Effective control of varroaosis using powdered thymol, Apidologie 22 (1991) 135–145.
- [3] Koeniger N., Fuchs S., Control of *Varroa jacobsoni* Oud. in honey bee colonies containing sealed brood cells, Apidologie 19 (1988) 117–130.
- [4] Moretto G., Gonçalves L.S., De Jong D., Michuette M.Z., The effects of climate and bee race on *Varroa jacobsoni* Oud infestation in Brazil, Apidologie 22 (1991) 197–203.
- [5] Moritz R.F.A., Mautz D., Development of *Varroa jacobsoni* in colonies of *Apis mellifera capensis* and *Apis mellifera carnica*, Apidologie 21 (1990) 53–58.
- [6] Rickli M., Imdorf A., Kilchenmann V., Treatment against varroaosis using compounds of essential oils, Apidologie 22 (1991) 417–421.
- [7] Sakofski F., Koeniger N., Fuchs S., Seasonality of honey bee colony invasion by *Varroa jacobsoni* Oud., Apidologie 21 (1990) 547–550.
- [8] Zar J.H., Biostatistical Analysis, Prentice Hall, Upper Saddle River, New Jersey, 1999.