# Monitoring Method as a Basis for Need-based Control of Varroa Mites (*Varroa jacobsoni*) Infesting Honey Bee (*Apis mellifera*) Colonies

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**Summary** — To avoid excessive use of pesticides in controlling varroa mites (*Varroa jacobsoni*) in honey bee (*Apis mellifera*) colonies, a method for monitoring the population size of the mites was developed. The relationship between the size of mite populations (y) in full-size honey bee colonies and natural mite mortality, measured as the number of mites falling on plastic inserts (drop-down), was investigated in Danish apiaries. The results suggest that a straight linear model (y = + x) describes the relationship between the mite population present in a colony and the calculated daily number of naturally dead mites collected on inserts during either 1-week or 3-week periods before sampling. The parameters of the straight line relationship between the population size and the daily mite drop-down during a 1-week period are: $\beta = 0.0069$ and $\alpha = -1.858$ ($r^2 = 0.77$, $p < 0.0001$). For a 3-week period, the parameters are: $\beta = 0.0063$ and $\alpha = -0.403$ ($r^2 = 0.83$, $p < 0.0001$). If the model input is adjusted for the brood-rearing pattern of the sampled colonies, i.e. colonies with capped brood cells covering less than one side of a comb in total (2800–3200 cells) are excluded from the input, the fit of the model is improved. In this case, the parameters for the 1-week sampling period are: $\beta = 0.0075$ and $\alpha = -1.184$ ($r^2 = 0.88$, $p < 0.0001$), and the parameters for the 3-week sampling period are: $\beta = 0.0071$ and $\alpha = -0.864$ ($r^2 = 0.91$, $p < 0.0001$).

*Key words: Varroa jacobsoni, Apis mellifera, honey bees, monitoring method, need-based control.*

## Introduction

The varroa mite (*Varroa jacobsoni* Oudemans; Acarina: Varroidae) is a severe parasite distributed worldwide in honey bee (*Apis mellifera* L.; Hymenoptera: Apidae) colonies (1). The mite was introduced into honey bee colonies in Denmark in 1984 (2), and is now widespread throughout the country (3). As damage to bee colonies increases with build-up of varroa mite populations in the respective colonies, an early control of the mites is necessary to prevent bee colonies from dying of secondary infections (4, 5). In many countries, varroa mites are controlled by pesticides. However, several studies have shown the presence of pesticide residues in wax and honey after pesticide treatment of honey bee colonies against varroa mites (6–11).

To avoid the excessive use of pesticides in controlling varroa mites in honey bee colonies through prophylactic application, attempts have been made to estimate total varroa population size in infested bee colonies by using non-destructive methods. The most effective non-destructive sampling method seems to be counting the number of dead mites dropping onto the bottom board of the beehives (12), but, according to Rademacher (13), neither the long-term (5 months) forecast of mite population development based on natural mite mortality, nor the mite population monitoring of an apiary, are possible. Furthermore, Milani (14) reported a poor correlation between mite population size and natural mite mortality in individual colonies, based on results from heavily infested colonies in the northern part of Italy. On the other hand, reports from similar experiments in Hohenheim, Germany (12, 15), with 1-week sampling of natural mite mortality, showed good correlation

with the total number of mites present in individual experimental bee colonies (nuclei). However, Fries *et al.* (16) reported that the model described by Liebig *et al.* (where mite population size can be calculated by multiplying daily natural mite mortality by a factor of 120 [12]) probably underestimates the population size, under Finnish conditions.

The aim of the present study was to examine the relationship between mite population size and natural mite mortality (measured as dead mites dropping onto the bottom board of a bee hive) in individual infested full-size honey bee colonies, under Nordic climatic conditions. The establishment of such a relationship will provide a basis for varroa mite control on the basis of need, thereby preventing prophylactic control treatments with pesticides or organic acids.

## Materials and Methods

The bee colonies were maintained in ten frame boxes with the Swedish standard frame (366 × 222mm). The queens were Danish strains of *A. mellifera ligustica spinola*, which are not resistant to varroa mites. The colonies were not treated with acaricides during the experiment.

Natural varroa mortality in 30 full-size honey bee colonies was sampled by counting naturally dead mites (drop-down) on plastic inserts (25 × 30cm) on the bottom board, underneath the brood nest of the colonies, as described by Fries *et al.* (16). In the period from May to October for 3 years, daily mite mortality was calculated, based on the number of mites accumulated in 1 week in 25 colonies. In addition, daily mite mortality was calculated, based on the total number of dead mites in all 30 colonies over 3 weeks. Successively throughout the sampling period, total numbers of mites were counted in sample bee colonies that were anaesthetised with $CO_2$ and killed by freezing. The number of capped brood cells in each colony at the point of termination was estimated visually, according to the method of Rogers *et al.* (17). The total number of mature female mites (distinguished by their light brown to brown colour) in the colonies were counted by using the method described by Ritter *et al.* (18). Briefly, the cappings of the sealed brood from a colony were removed and the mites were washed from the larvae and pupae by using a hand-shower over a double wire screen. To separate mites from the adult bees, the bees were first shaken in 70% ethanol for 3 to 4 minutes, and then washed in the same way as the brood (16).

The naturally dead mites caught on the inserts (drop-down) were assumed to be Poisson distributed, and linear regression analysis was used to investigate the relationship between natural mite mortality, measured as daily mite drop-down during periods of 1 or 3 weeks, and the absolute mite population size in the colonies.

Since varroa mites are only able to reproduce in capped bee brood cells (4, 19), population estimates might be improved if a relationship between population size and capped brood exists. Therefore, investigations on the influence of the amount of capped brood on the total mite population were conducted, as well as investigations into the relationship between the mite population on adult bees and brood, respectively, within each colony.

## Results

Regression analysis (20) suggested a significant relationship between the number of mites on adult bees and in the capped brood cells in the individual bee colonies; however, the correlation was poor ($p < 0.0001$, $r^2 = 0.40$, $n = 30$). The number of mites on the adult fractions of the bee populations did not relate to the amount of brood present in the respective bee colonies (Spearman rank [20], $r_s = -0.317$, $p = 0.088$, $n = 30$), nor did the number of mites in the larval fractions of the populations (Spearman rank, $r_s = 0.319$, $p = 0.086$, $n = 30$). Successive sampling showed no correlation between the size of the varroa populations in relation to time of year in the season May to October (Spearman rank, $r_s = 0.007$, $p = 0.972$, $n = 30$).

The calculated daily mite mortality in the 30 bee colonies, measured as mite drop-down, ranged from 0 to 68 mites a day, and the total number of mites in the colonies ranged from 22 to 10,327 mites per colony. The results suggest that a straight linear model ($y = \alpha + \beta x$) describes the relationship between the mite population present in a colony (x) and the calculated daily number of naturally dead mites (y) collected on inserts during either 1-week or 3-week periods before the sampling date. Regression analysis gave the following parameters of the model for a 1-week period: $\beta = 0.0069$ (± 0.0008 SE) and $\alpha = -1.858$ (± 2.614 SE), $r^2 = 0.77$, $p < 0.0001$, $n = 25$; Figure 1). For a 3-week period, the parameters were = 0.0063 (± 0.0005 SE) and $\alpha = -0.403$ (± 1.62 SE), $r^2 = 0.82$, $p < 0.0001$, $n = 30$; Figure 2). However, when the model input was adjusted for the brood rearing pattern of the sampled colonies, i.e. colonies with capped brood cells covering less than one side of a comb in total (2800–3200 cells) were excluded from the calculations, the fit of the model was improved. The parameters for 1-week sampling were then: $\beta = 0.008$ (± 0.0008 SE) and $\alpha = -1.184$ (± 2.708 SE), $r^2 = 0.88$, $p < 0.0001$, $n = 15$; Figure 3), and

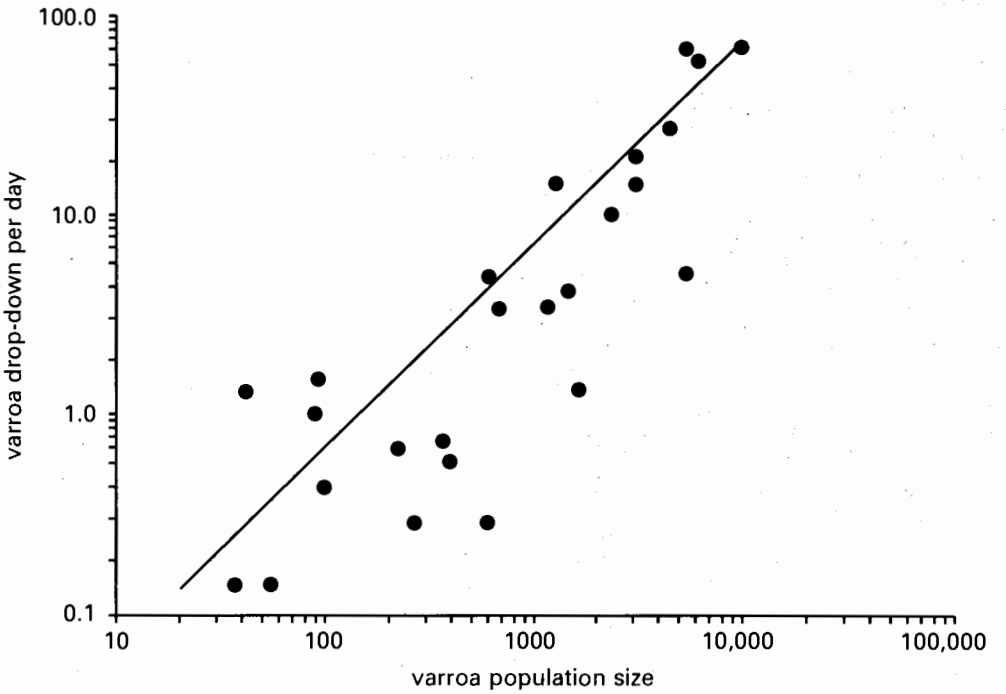
the parameters for a 3-week sampling period were then: $\beta = 0.007$ (± 0.0006 SE) and $\alpha = -0.864$ (± 2.029 SE), $r^2 = 0.91$, $p < 0.0001$, n = 15; Figure 4).

## Discussion

The distribution of varroa mite populations on brood and adult bees, respectively, showed no clear relationship (the fit of a linear model was only 40%). This corresponds with the findings of Fuchs & Koeniger (21), who suggested that samples must be taken from both adult bees and brood, in order to estimate total mite population in bee colonies based on bee sampling. No seasonal variation was observed in the present study, indicating that the varroa mite drop-down sampling method can be used throughout the season May to October.

The present study suggests that, by counting naturally dead varroa mites that fall onto inserts at the bottom of bee hives in a period of 1, or preferably 3, weeks in Danish honey bee colonies, it is possible to estimate the absolute number of mites in the respective colonies with a straight-line model. These findings correspond with findings from Germany (12), although the parameter estimates in the present model differ significantly from the German estimates. If the German parameter estimates are used on the drop-down figures of the present study, the model underestimates the absolute mite population size observed in Denmark. This difference in parameter estimates could be because the study of Liebig *et al.* (12) was

**Figure 1: The relationship between varroa mite population size and calculated daily natural mite mortality during a 1-week sampling period**

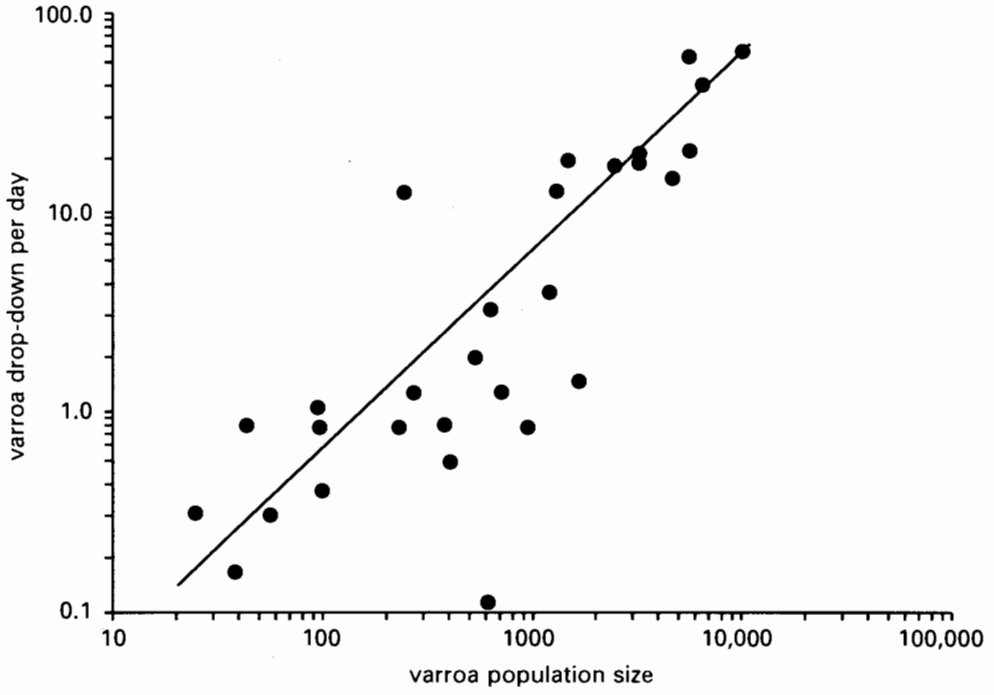



*The straight line indicates best fit of a linear regression on non-transformed data ($r^2 = 0.77$, $p < 0.0001$).*

performed on nuclei with newly introduced mite populations, rather than on full-size bee colonies with mite populations with a stable age distribution.

Rademacher (13) found no clear relationship between natural death-rate in summer and early autumn and infestation level in late autumn. However, Rademacher (13) included a high number of non-pigmented mites on the inserts in the death-rate estimations. These non-pigmented mites are not fully mature at the time of emergence of the infested bees and will therefore die shortly after bee emergence (22). These immature mites were not included in the death-rate estimations in our study or in a model study by Omholt & Crailsheim (22). Omholt & Crailsheim were able to establish a death-rate/infection-level relationship by developing a dynamic model based on various published studies on varroa population dynamics. The model showed that prediction of the degree of varroa mite infestation by means of natural death-rate data might be possible, provided that these data are interpreted in relation to the brood-rearing pattern of the infested colonies. The present study supports the results of Omholt & Crailsheim (22), as the correlation coefficients in our experiment increase to 91% when broodless colonies are excluded from the model input. In broodless colonies, all mites must stay on adult bees, of which a large proportion spend a large part of the day outside the hive. Hence, a substantial proportion of the naturally dying mites must

**Figure 2: The relationship between varroa mite population size and calculated daily natural mite mortality during a 3-week sampling period**

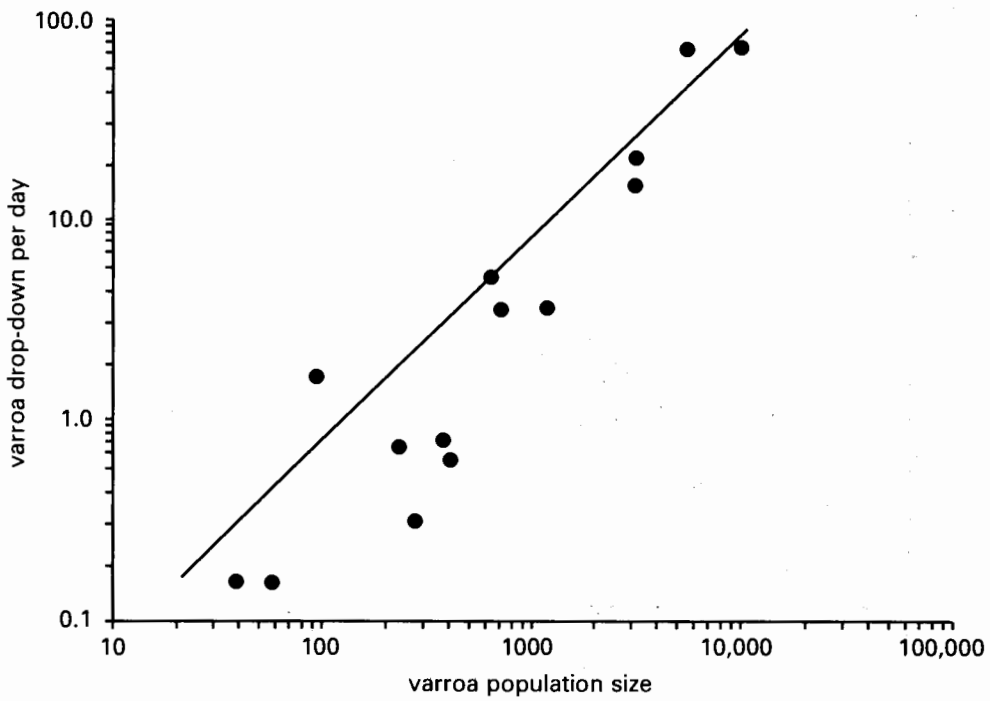



*The straight line indicates best fit of a linear regression on non-transformed data ($r^2 = 0.83$, $p < 0.0001$).*

drop off the bees outside the hive. The natural mite mortality will, under such circumstances, be underestimated based on drop-down on bottom board inserts. Furthermore, as mites only reproduce on the capped brood, the mite population in broodless colonies will not have a stable age distribution, so the Poisson distribution assumption is likely to be wrong. The results of our experiment suggest a simple quantification of the brood-rearing pattern as the overall amount of capped brood cells per individual bee colony. The confidence of the monitoring model will increase, if the model is only applied to colonies with capped brood cells covering more than one side of a comb in total (2800–3200 cells). Furthermore, our study suggests that the confidence of the model will not be further improved by increasing that input constraint.

The treatment threshold for varroa mites in a temperate climate is approximately 1000 mites/colony in the spring. If action is not taken at that infestation level, the population will exceed 10,000 mites/colony by the autumn, and the colonies will then die (23). As there is a high correlation between the actual mite population size and natural varroa mortality, measured as cumulative drop-down on inserts over a 3-week period, this model can be used as a decision-making sampling tool in the brood-rearing period, by using varroa drop-down to monitor population size. For example, if a daily drop-down of seven mites is calculated, the model predicts a total varroa population size in the

**Figure 3: The relationship between varroa mite population size and calculated daily natural mite mortality, adjusted for the brood-rearing pattern in a 1-week sampling period**




*The straight line indicates best fit of a linear regression on non-transformed data ($r^2 = 0.88$, $p < 0.0001$).*

colony of 941 mites (± 147, 95% prediction interval), and control measures should be initiated. However, proper validation of the model is necessary before it can be generally recommended to bee-keepers.

After successful validation, the present study will make a contribution to the reduction of use of pesticides in honey bee colonies. Thus, the study provides a necessary element in the effort of changing the present control strategy, which is currently based on prophylactic treatments with acaricides, to control strategies based on need. Furthermore, the model is essential for alternative, non-pesticide treatments that are generally so labour intensive that they are only operational on a need-based basis.

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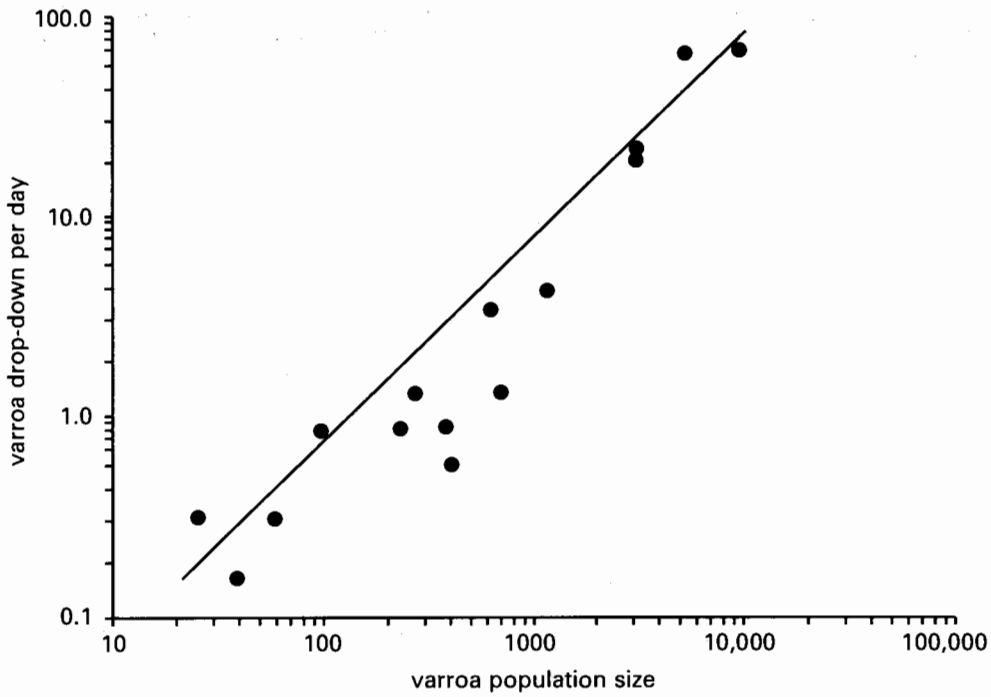
**Figure 4: The relationship between varroa mite population size and calculated daily natural mite mortality, adjusted for the brood-rearing pattern in a 3-week sampling period**




*The straight line indicates best fit of a linear regression on non-transformed data ($r^2 = 0.91$, $p < 0.0001$).*

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