

A comparative evaluation of sampling methods for *Varroa destructor* (Acari: Varroidae) population estimation*

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Abstract – Obtaining a precise estimation of *Varroa destructor* population size in honeybee colonies is an important tool to implement integrated control practices. Therefore, it is necessary to know the adequacy and accuracy of the sampling methods used. We studied three sampling methods simultaneously to estimate the size of mite population: killing the mites with an acaricide, estimating the infestation level in adult bees and brood cells, and sampling the natural mortality of the mite. The three methods were compared pairwise by regression analysis. A good linear relationship was found between the three methods. Sampling the natural mortality was a reliable method for estimating absolute population size as long as some conditions are satisfied (colonies must be broodright and not collapsing). A mathematical equation is determined to translate the mite's natural death rate into absolute population numbers.

Varroa destructor / *Apis mellifera* / population size / sampling method / natural mortality

1. INTRODUCTION

Accurate sampling methods are required to estimate the population size of *Varroa destructor* Anderson and Trueman in honeybee colonies. The three methods most often used are: (1) killing the mites in the colony by chemical products; (2) estimating the mite population by sampling immature (drone and worker brood cells) and adult bees; and (3) sampling the natural death rate of the mite.

One of the most reliable methods to estimate the mite population is to kill all the *V. destructor* in a colony using chemical products. Dead mites are collected on floorboard traps and counted. Theoretically the method gives an absolute population estimate but in reality is

not exact because no chemical treatment guarantees 100% mite kill. However, since the efficacy of the acaricides used such as Apistan is very high, above 95%, and has small variability (Ferrer-Dufol et al., 1991), we may accept it as an absolute population estimate, considering that no resistance to the acaricide is observed. As the method is destructive, however, it cannot be used in a periodical sampling experiment.

V. destructor population size can also be estimated by sampling the average number of adult mites infesting adult bees and brood cells. The method can be accurate only if a large number of adult bees and brood cells are examined. Moreover, the reliability of both samples used separately is not very high; a further increase in reliability may be achieved by combining a brood sample and a bee sample (Fuchs, 1985). At least 200 bees and brood cells should be examined to obtain reliable figures. Some authors advise taking even larger samples considering that the sample size should be

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inversely related to the infestation level; i.e., to the probability of finding an infested cell (Ritter and Ruttner, 1980; Pappas and Thrasivoulou, 1988). Nevertheless, some statistical sampling techniques may improve the estimation of brood infestation without requiring such large samples (Floris, 1997; Reich et al., 1998). Sampling bees and brood provides relative estimates of the mite population, but this method can be used to obtain absolute population estimates if estimates of the total numbers of adult workers and of immature bees are available.

V. destructor natural mortality can be estimated periodically in any beekeeping management using appropriate floorboard traps. The method gives only a relative estimate of mite population size, but considering it can be broadly used from an integrated control management standpoint, it is of important practical interest to know whether it is possible to convert the natural mortality of the *V. destructor* into absolute population numbers. The correlation between the natural mite mortality and the total number of mites in the colonies has been a subject of study by several researchers with different conclusions; some authors found a good linear relationship while others did not (Rademacher, 1985; Imdorf and Kilchenmann, 1990; Milani, 1990; Calatayud and Verdu, 1993; Garza and Wilson, 1994; Brødsgaard and Brødsgaard, 1998).

It is important to analyse how different methods of estimating *V. destructor* population size agree and to check their reliability. Such an analysis would provide us with simultaneous information concerning the quality of the data and statistical tools to convert relative estimates into absolute population numbers. In this study, three sampling methods were used and their results compared. A mathematical equation to translate the natural mortality of the *V. destructor* mite into absolute population in the colony was also developed. Furthermore, the necessary conditions to sustain a good linear relationship between the mite population size and its natural mortality were analysed and a synthesis to integrate contributes of different authors on this subject is provided.

2. MATERIALS AND METHODS

Three sampling methods were used to estimate the mite population size in 22 honeybee colonies:

monitoring the natural mortality of mites; the mites infesting adult bees and brood cells; and the mites killed by chemical treatments. The honeybee colonies (considered to be *Apis mellifera iberica*) were kept in "Lusitana" beehives located near Lisbon. Experiments were conducted during summer and autumn in two years, 1994 and 1995. During the experimental periods the daily average temperature ranged from 25 °C to 11 °C. The amount of sealed worker cells in the experimental colonies was on average (\pm SE) $6\,006 \pm 429$ and $10\,400 \pm 1033$ in the first and second year, respectively.

The natural mortality of *V. destructor* was estimated by means of a floorboard trap. The floors of the hives were set up with a 3 mm wire mesh in a wooden frame with a tray under it acting as a removable drawer. All the litter that fell into the floorboard trap was collected weekly and the dead mites were counted. A sheet of paper was placed inside the drawer and replaced each week. To prevent ants from entering the floorboard trap and preying on the mites, the borders of the sheets of paper were covered with "Napvik" a sticky material, and the stands for the hives were adapted with oil traps.

Adult bees for sampling mite infestation were taken directly from the combs of the hives into a jar. About 200 adult bees were taken from each colony. To have a representative sample, between 4 to 6 frames were chosen both from the brood chamber and the honey super. Samples were taken during the day. In the laboratory, the bee samples were anaesthetised with ether. Bees were then repeatedly washed with water and detergent and separated from mites through a 3 mm mesh. The washing process was repeated until no more mites were found on the bees. The separated mites were finally counted.

For sampling mites on worker and drone brood cells, two frames with recently sealed brood (pupae were not older than the purple to dark-purple eye stage) were selected from each colony and examined in the laboratory. The frames were returned to the colonies immediately after being examined. Four sample units of 50 sealed worker cells each were randomly selected from the two brood frames using a numbered grid. Each sealed cell was uncapped, the pre-pupa or pupa inside it was carefully examined and adult female mites on it were counted. The walls of the cells and removed caps were also examined as the mite frequently hides there. In combs with drone cells ($n = 29$) since only a few drone cells were observed (on average 9.2 ± 1.43 SE) all the sealed drone cells were also examined.

At each sampling date, the number of adults and sealed brood cells in the experimental colonies were also estimated to obtain absolute population figures for *V. destructor*. Adult bee populations were estimated comparing the frames with bees with calibrated photographs. Bees foraging in the field were

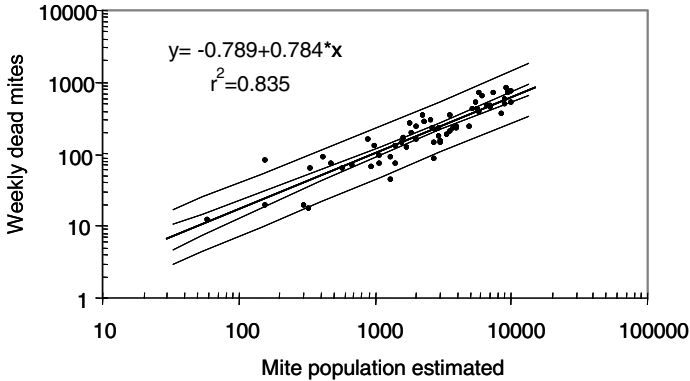


Figure 1. Comparison of the weekly natural mite mortality and total mite population estimated through the adult bees and pupa infestations. The solid line represents the point estimates on log-transformed data and the inner and outer dotted lines are 95% confidence and prediction limits respectively.

also considered by estimating the average flight time and counting the number of bees entering into the hives during that period. Sealed worker cells were counted using video camera recording. The absolute number of mites in a colony was calculated by multiplying the sum of adult, worker and drone pupae numbers by the respective average number of mites per adult bees and sex of pupa estimated.

The 22 colonies were monitored and their mite infestation levels on adult bees and brood cells were also sampled before treatment with an acaricide, APISTAN® strips, both in November 1994 and December 1995. In the treated colonies the mites killed by the acaricide were collected weekly in floorboard traps and counted during six successive weeks. The total number of mites collected was considered to equal the mite population in the colony before treatment. No resistance by the mite to fluralinate was observed during the experimental period.

To obtain a more representative sampling range, the numbers of mites on adult bees and worker and drone sealed brood cells were also estimated at five periods, over 21-day intervals between August and November 1994 in 6 colonies selected at each sampling period from the 22 experimental colonies. The selection criterion for the colonies was that they display a range of low, medium and large infestation levels to allow for a large interval of variation of the mite population size. Within each infestation group, two colonies were randomly selected.

Comparison of data obtained from the three sampling methods was performed using regression analysis. The statistics PRESS and $R^2_{\text{prediction}}$ (Montgomery and Peck, 1982) were used to evaluate the predictive performance of the linear regression model to translate naturally weekly dead mites into mite absolute population. The reproducibility of the measurements of the mite population size achieved through the different methods was evaluated by esti-

imating the concordance correlation coefficient (Lin, 1989, 1992).

3. RESULTS

The absence of brood, which occurred in some colonies due to queen supersedure, highly influenced the mite mortality rate. It was observed that the natural mite mortality increased greatly during the broodless period, particularly in the first weeks. In contrast, when brood rearing resumed the weekly count of dead mites decreased greatly due to the invasion of many phoretic mites into the brood cells where they remain protected for almost two weeks. The broodless colonies were outliers, falling more than three standard deviations from the average of the residuals, and caused a large decrease of fit in the regression analysis. Therefore, four cases in which colonies were superseding the queens were removed from the analysis. Colonies in a terminal stage of collapse due to varroosis also behaved differently because of their very low bee population size and variable parasite population levels. Seven cases observed in these circumstances were also found to be outliers and removed prior to analysis. Consequently, our analysis was only valid for colonies with brood and that were not collapsing.

Figure 1 shows the relationship between the weekly average mite mortality observed during the last two weeks, and the absolute mite population estimated from the adult bee and pupal infestations. We considered the average number of dead mites observed over the last two weeks assuming that the number of mites found in the

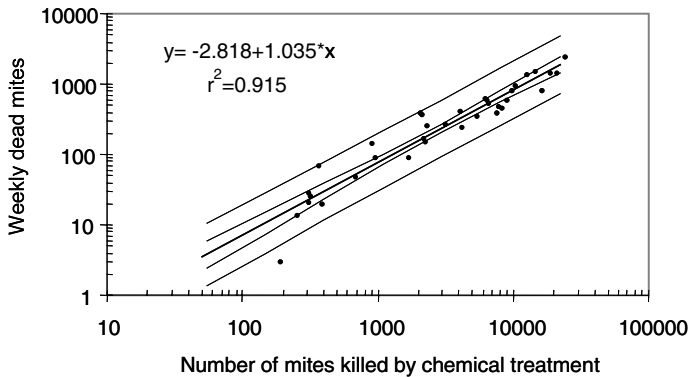


Figure 2. Comparison of the weekly natural mite mortality and total number of mites killed by the chemical treatment (accumulated data from the years 93 and 94). The solid line represents the point estimates on log-transformed data and the inner and outer dotted lines are 95% confidence and prediction limits respectively.

brood cells was a function both of the mite population occurring in that week and in the week before. Sixty-three observations were considered for this analysis, from 22 colonies in the first year, 22 colonies in the second year plus 30 observations taken during August to November 1994; 11 outliers were removed. Since a preliminary data analysis showed an increasing variance with the increase number of mites a log transformation of the variables was used to take into account the non-constant error variance ($r^2 = 0.835$). The residuals of the model were normally distributed and independent, the first order autocorrelation estimation was $r = -0.042 (\pm 0.126 \text{ SE})$.

The average mite mortality rate was determined by dividing the number of naturally dead mites by the absolute mite population estimate. The relationships between mite death rate and both mite population and the ratio of mites to worker pupae, were studied by linear regression analysis. Pearson correlation coefficients between mite death rate and the absolute mite population and between mite death rate and the ratio of mites to worker pupae were very low: $r = -0.35$ and -0.279 , respectively. Non-linear relationships were also studied but had no better fits. This result supports the hypothesis that in broodright and not collapsing colonies, the death rate of *V. destructor* is independent of its population density. If we consider this mortality rate constant, it is then possible to convert relative estimates of the mite natural mortality into absolute population estimates by a simple linear equation.

The weekly counts of natural mite mortality in the week before the chemical treatment, 22 observations in the first year plus 22 obser-

vations in the second year, were also compared with the total numbers of mites killed by chemical treatments ($r = 0.951$). Nine outliers were removed. A simple linear regression between these two variables followed the equation $y = 0.0803x + 8.889$, which corresponded to a daily mite mortality rate of about $0.011 (\text{day}^{-1})$. Yet, again a log transformation of the variables was needed to correct a non-constant error variance (Fig. 2). For this model the residuals were normally distributed and independent; the first order autocorrelation estimation was $r = -0.068 (\pm 0.169 \text{ SE})$.

The better fit in this analysis suggested that the mortality counts provided more accurate data of the mite population size. To estimate the total mite population (M) from the natural mite mortality (D_m) we may then use the regression equation $\log(D_m) = -2.818 + 1.035 \log(M)$. The standard errors of the coefficients (β_0, β_1) were, respectively, 0.439 and 0.054. The PRESS statistic was 8.262, and the $R^2_{\text{prediction}}$ was 0.901, so the model may be considered to have a good predictive performance.

According to biological criteria in the regression analysis, the number of naturally dead mites (D_m) was the dependent variable. However, when it is convenient to consider the absolute mite population as the dependent variable, for estimation purposes, then the equation $\log(M) = 3.159 + 0.889 \log(D_m)$ may be used with the same fit and predictive qualities as the former model. The concordance correlation coefficient between the mite absolute population size estimated by this equation and the total number of mites killed by chemical treatment was also reasonably good ($r_c = 0.932$).

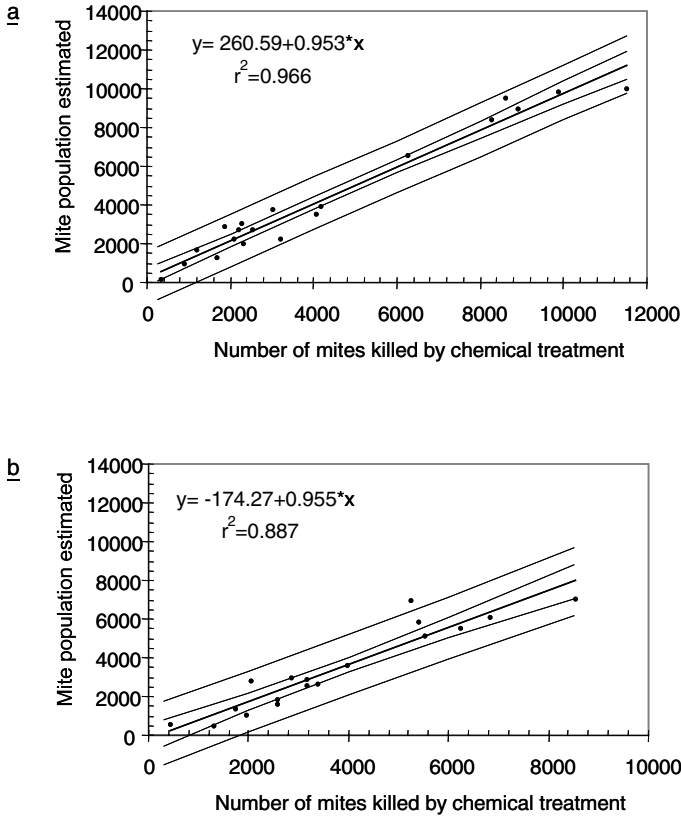


Figure 3. Comparison of the mite population estimated from the adult bee and brood cells infestations and the overall number of mites killed by the chemical treatment. The solid line represents the point estimates and the inner and outer dotted lines are 95% confidence and prediction limits respectively. (a) Data from November 1994. (b) Data from November 1995.

supporting a good reproducibility of the two methods.

Finally, the total number of mites killed by the chemical treatments was also compared with the absolute mite population estimated from the infestation levels sampled in adult bees and brood cells immediately before the chemical treatment. Two separate analyses were performed for the years 1994 and 1995, respectively (Fig. 3). Since we compared two estimates of the absolute population, this comparison allowed us to check the reliability of the data.

The regression analysis of the total number of mites killed by the treatment versus the number of mites estimated on brood cells and adult bees exhibited a very good linear relationship. In particular, in the first year both the coefficient of correlation ($r = 0.983$) and the concordance correlation coefficient ($r_c = 0.933$) showed that these two variables were similar; that is, a good reproducibility between

the two methods was observed. In the second year, the linear relationship and the reproducibility were still good, although not as high ($r = 0.942$, $r_c = 0.878$, respectively). A good linear relationship between the two methods was still observed when the two years were considered simultaneously by an ANCOVA analysis (Square multiple $R = 0.944$). Also the difference between the two years was not statistically significant ($P = 0.093$). These results can be considered particularly good if it is considered that there are many accumulated errors in achieving the absolute mite estimation through adult and immature bees' samples.

4. DISCUSSION

In this study, three methods were analysed to evaluate the mite population: (1) the weekly counts of natural mite mortality, (2) adult and pupal infestation levels, and (3) the total

number of mites killed by a fluvalinate treatment.

Estimating the mite population through adult and pupal infestations proved to be a very precise method. The absolute mite population was estimated by a combination of adult bee and brood samples and compared to the number of mites killed by chemical treatment. This relationship was high in the first year ($r^2 = 0.966$) and still good in the second year ($r^2 = 0.887$), which indicated that the data were highly reliable. However, the method was very demanding in the field and laboratory to estimate, simultaneously, infestation levels on adults and brood cells and the bee population size in the colony. Moreover, the method is destructive, so it cannot be used periodically to survey the mite population growth. During a period of about five months after chemical treatment, this sampling method was tried occasionally in a few colonies and usually revealed no mites or a negligible number, despite there being an increasing mite population in those honeybee colonies. As is evident the method is not appropriate for low infestations, with less than 2% of mites in brood cells, unless very large samples are taken.

Sampling natural mite mortality is an easy way of estimating the mite population numbers without interfering with the colony or the mite itself. One criticism is that it is an unreliable method because factors other than population size can affect mite mortality.

Several researchers have studied the correlation between the natural mortality of *V. destructor* and its absolute population size in the colonies. Rademacher (1985) and Milani (1990) found variable results, which suggest that the natural death rate is not a reliable method for estimating the mite population. However, significant correlations between these two variables were found in other studies (Imdorf and Kilchenmann, 1990; Calatayud and Verdu, 1993; Brødsgaard and Brødsgaard, 1998). In the present study, a good linear relationship between the numbers of naturally dead mites and the total mite population of the colony estimated by the other two methods was also found. However, to achieve a high reliability, some conditions had to be observed, such as: (1) the method gave good results only for broodright colonies; (2) it was not adequate when colonies were collapsing from varroosis;

and (3) the method can only be used to estimate the existing absolute population, it is not reliable to predict a future population. When these conditions were satisfied, there was a high correlation coefficient when the weekly natural mite mortality was compared with the mites killed by chemical treatment, ($r^2 = 0.915$). Considering the condition (1) our finding is consistent with the work of Brødsgaard and Brødsgaard (1998) where the fit of the model was improved when colonies were selected with a minimum of about 3 000 brood cells.

In the work of Rademacher (1985), condition (3) was not held. This author related the natural death rate at one date with the mite infestation after about 1-month. However, this relationship does not depend only on the initial infestation level; it also depends on the sequential development of the mite population between the two dates, which introduces further variability. This is particularly relevant in cold climates during autumn when colonies cease brood rearing as shown in a simulation model proposed by Omholt and Crailsheim (1991). In the same way this explains why the author found a reasonably good correlation between the natural mite mortality in July and the population size in August/September ($r = 0.8$) while the correlation between the natural mite mortality in September and the infestation in October was very variable and low $r = 0.27$ – 0.71 .

Milani (1990) found that the correlation between the number of mites that fell daily and the number of mites in the colony captured after chemical treatment was very weak ($r = 0.10$ – 0.27). In this work all the colonies were severely infested, with mite populations ranging from 4 000 to 12 000. We observed that at this high level of infestation, the colonies start to collapse, brood starts to die in large numbers, and this affects the natural mortality rate of the mite, which becomes higher and more variable. If data in this phase is collected, the relationship between the two variables will be affected by this phenomenon and will be less reliable.

Also, there is a large variability in the daily mite mortality that may be mostly due to daily emerging bees with different infestation levels since the distribution of the mite among the brood is not uniform (Fuchs, 1988). This would explain why Garza and Wilson (1994) found greatly variable results using sticky boards

during 24 hours with an average mortality rate of 0.016 (day^{-1}) in January, 0.11 (day^{-1}) in April and 0.45 (day^{-1}) in July and a moderate correlation coefficient with the absolute population. We found that a more reliable relationship between the mite death rate and the mite population requires at least one or even two weeks of accumulated data. In addition, we might consider that the floorboard trap device may also capture living mites that fall naturally to the bottom of the colony and can not climb up. This is irrelevant considering that these mites are proportional to the naturally dead ones. However, this fact might be significant in the presence of factors which might cause additional mite fall, such as unusual disturbed colonies or the use of specific methods which induce the mite fall.

With this work we emphasise that observing the natural mortality of the population of *V. destructor* in honeybee colonies is a very practical method which can give reliable estimates of the mite population. Logically, this method relies upon the presumption that the mortality rate of the mite population is density-independent. If this condition is real then it is possible to convert relative estimates of the mite natural mortality into absolute population estimate by a simple linear equation. Our results support that this hypothesis is correct only if some conditions are observed, which also explains why some researchers showed a good correlation between the mite natural mortality and the mite population while others did not. The present study supports that the mite death rate only behaves in a density-dependent manner when infestations are very high, with a mean ratio of mite to worker brood greater than 1, which corresponds to a phase of colony collapsing with visible symptoms. To analyse the density-independent hypothesis we compared the mite death rate with the absolute mite population and with the ratio of mites to worker brood using the Pearson correlation coefficient. The results were very poor $r = -0.350$ and -0.279 , respectively, which emphasises the independence of the mite death rate from the population density.

In these circumstances the mean daily rate of mite mortality was approximately of 0.011 (day^{-1}). Calatayud and Verdu (1993) found a regression equation of $y = 5.8x + 7.9$ to translate the total number of mites that naturally died

over 65 days and the number of mites found after chemical treatment. This equation would give approximately a daily mite death rate of 0.0027 (days^{-1}) if we consider that the mite population over the experimental period remained constant. However during the 65 days of data collection the mite population was growing, according to the authors, at an intrinsic growth rate of increase, $r = 0.027$ (days^{-1}), so the real mite death rate should have been about 0.009 (days^{-1}) which is near our result. Also Imdorf and Kilchenmann (1990) analysed the correlation between the natural mortality and the mite population killed with an acaricide two months after. In this study the slope of the regression line was about 0.002, which corresponds, considering the above intrinsic growth rate, to a mite death rate of 0.010 (days^{-1}).

We admit that differences in the mite mortality rate may be a function of the race of bee (Moritz and Mautz, 1990; Ruttner and Hänel, 1992), and climatic conditions. In temperate climates an increase in mite longevity during winter has been reported. Martin (1998) reported a daily mite mortality of 0.006 in spring/summer when brood was present and a daily mite mortality of 0.002 in winter when colonies were broodless. The first value, for colonies with brood, is lower than the values mentioned above and is close to the result found by Brødsgaard and Brødsgaard (1998) (i.e. 0.0071–0.0075 days^{-1}) which may be explained by the race of the bee. Yet, in another part of his article Martin (1998) states that the mite population can be estimated when brood is present by multiplying the daily mite drop by 20–40. This figure corresponds to a daily mite mortality rate of 0.05–0.025 (days^{-1}), which is a wide range with considerably higher values.

In conclusion, considering the reasonably high reliability and facility of application, sampling the natural mite mortality is considered an excellent method to be used periodically. Like other works (Maul et al., 1988; Gerig, 1989; Imdorf and Kilchenmann, 1990; Sammataro et al., 2002) this study supports the view that this sampling method is adequate for use in an integrated pest management (IPM). The method is reliable for monitoring the mite population and so is a decisive tool for avoiding blind chemical treatments. Effectively, the increasing resistance of *V. destructor* to acaricides (Mozes-Koch et al., 2000; Floris et al., 2001;

Spreafico et al., 2001; Elzen and Westervelt, 2002) and the problems with acaricide residues in honey and bee wax (Korta et al., 2001) justify such effort toward IPM strategies. Meanwhile, considering differences in the mite mortality rate due to bee race or climatic conditions, the method relies upon the estimation of this parameter for each particular race or region.

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Résumé – Comparaison de méthodes d'échantillonnage pour estimer les populations de *Varroa destructor*. Trois méthodes d'échantillonnage pour estimer la taille des populations de l'acarien *Varroa destructor* ont été comparées sur deux ans dans 22 colonies d'abeilles domestiques (*Apis mellifera*) : (1) destruction par le fluvalinate de tous les acariens présents dans la colonie. On a considéré que le nombre total d'acariens morts récoltés durant six semaines consécutives équivalait à la population d'acariens de la colonie avant le traitement ; (2) estimation de la population d'acariens par échantillonnage d'abeilles immatures (200 cellules de couvain d'ouvrières operculé et toutes les cellules de mâles operculées) et d'adultes ($n = 200$). On a estimé le nombre total d'abeilles immatures et adultes pour calculer le nombre total d'acariens dans la colonie ; et (3) échantillonnage de la mortalité naturelle de la population d'acariens en dénombrant chaque semaine les acariens trouvés morts sur un plateau de fond « anti-varroas ». Les méthodes 2 et 3 ont été en outre appliquées à six colonies choisies au hasard lors de cinq échantillonnages supplémentaires. Les trois méthodes ont été comparées deux à deux par une analyse de régression. Il y a eu une bonne relation linéaire entre le nombre d'acariens morts naturellement chaque semaine et la population absolue estimée par les infestations des nymphes et des adultes ($r^2 = 0,835$) (Fig. 1). Les données sur les acariens morts naturellement chaque semaine (D_m) ont été comparées avec le nombre total d'acariens tués par traitement chimique (Fig. 2). La régression donne une équation qui transforme D_m en population absolue d'acariens (M) : $\text{Log}(D_m) = 2,818 + 1,035 \log(M)$. Le modèle a une bonne adéquation avec les données ($r^2 = 0,915$) et de bonnes performances de prédiction ($R^2_{\text{prédiction}} = 0,901$). Le coefficient de corrélation des concordances confirme la bonne reproductibilité des deux méthodes ($r_c = 0,932$). Enfin, le nombre total d'acariens tués par traitement chimique a été comparé à la population absolue

d'acariens estimée d'après les niveaux d'infestation des abeilles adultes et des cellules de couvain échantillonnées juste avant le traitement chimique (Fig. 3). La première année tout particulièrement, le coefficient de corrélation ($r = 0,983$) et le coefficient de corrélation des concordances ($r_c = 0,933$) montrent tous deux que ces deux variables sont semblables.

Nous en concluons qu'estimer la population absolue d'acariens par les infestations des abeilles peut être une méthode très précise si l'on utilise à la fois des échantillons de couvain et d'abeilles adultes. La méthode nécessite néanmoins un trop gros travail de terrain et de laboratoire pour estimer simultanément les niveaux d'infestation des adultes et des cellules de couvain et le nombre d'abeilles dans la colonie. En outre elle est destructive et ne peut donc pas être répétée. Par contre, l'échantillonnage des acariens morts naturellement constitue une façon aisée d'estimer la population d'acariens sans interférer avec la colonie ou l'acarien lui-même. Dans cette étude il y a eu une bonne corrélation entre le nombre d'acariens morts naturellement et la population absolue estimée par les deux autres méthodes. Mais la méthode n'a été valable que pour les colonies qui ont du couvain et ne s'effondrent pas à cause de la varroose. Il faut aussi cumuler le nombre d'acariens morts sur une semaine au moins.

Varroa destructor / *Apis mellifera* / taille de la population / méthode d'échantillonnage / mortalité naturelle / étude comparative

Zusammenfassung – Eine vergleichende Bewertung von Beprobungsmethoden zur Populationsbestimmung von *Varroa destructor* (Acari: Varroidae). Zum Vergleich der Ergebnisse wurden zwei Jahre lang drei verschiedene Beprobungsmethoden zur Bestimmung der Populationsgröße von *Varroa destructor* an 22 Bienenvölkern durchgeführt: (1) Alle Milben wurden in den Völkern mit Fluvalinat abgetötet. Als Milbenpopulation vor Behandlung wurde die Gesamtzahl der an 6 aufeinanderfolgenden Wochen gezählten toten Milben gewertet; (2) Die Milbenpopulation wurde durch das Sammeln von Entwicklungsstadien (200 verdeckelte Arbeiterinnenzellen und alle verdeckelten Drohnenzellen) und von 200 adulten Bienen bestimmt. Zusätzlich wurde die Gesamtmenge der Brut und adulter Bienen in den Völkern ermittelt. Hierdurch konnte die Gesamtzahl der im Volk vorhandenen Milben rechnerisch abgeschätzt werden; (3) Die natürliche Milbenmortalität wurde durch wöchentliches Auszählen von Bodeneinlagen ermittelt. Bei 6 zufällig ausgewählten Völkern wurden Methoden (2) und (3) an 5 zusätzlichen Sammelterminen durchgeführt. Die drei Methoden wurden paarweise mit einer Regressionsanalyse verglichen. Es bestand eine gute lineare Beziehung zwischen dem natürlichen wöchentlichen Totenfall und der aus dem Befall der Adultbienen und der verdeckelten Bienenbrut ermittelten absoluten

Milbenpopulation ($r^2=0,835$, Abb. 1). Der wöchentliche natürliche Totenfall wurde mit der Gesamtzahl der durch die chemische Behandlung getöteten Milben verglichen (Abb. 2). Aus der Regression wurde eine Umrechnungsformel zur Bestimmung der absoluten Milbenpopulation (M) aus dem wöchentlichen Totenfall (D_m) entwickelt: $\log(D_m) = -2,818 + 1,035 \log(M)$. Dieses Berechnungsmodell zeigte eine gute Übereinstimmung ($r^2=0,915$) und eine gute Vorhersagegenauigkeit ($R^2_{\text{prediction}} = 0,901$). Weiterhin unterstützte der hohe Konkordanzkorrelationskoeffizient die gute Reproduzierbarkeit der beiden Methoden ($r_c = 0,932$).

Zuletzt wurde die Gesamtzahl der durch die chemische Behandlung getöteten Milben mit der absoluten Milbenpopulation verglichen, wie sie aus den unmittelbar vor der Behandlung entnommenen Proben von Adultbienen und verdeckelter Brut errechnet worden war (Abb. 3).

Insbesondere im ersten Versuchsjahr zeigten sowohl der Korrelationskoeffizient ($r = 0,983$) als auch der Konkordanzkoeffizient ($r_c = 0,933$), dass diese beiden Variablen sich sehr ähnlich verhielten. Daraus schlossen wir, dass die absolute Milbenpopulation über den Befall der Bienen mit einer Kombination von Brutproben und Bienenproben sehr genau abgeschätzt werden kann. Allerdings erfordert diese Methode wegen der gleichzeitigen Erfassung des Bienen- und Brutbefalls sowie der Anzahl von Brutzellen und Bienen in den Völkern einen zu hohen Arbeitsaufwand im Freiland und Labor. Darüber hinaus ist die Methode destruktiv und kann nicht wiederholt zur Anwendung kommen.

Demgegenüber ist das Sammeln des natürlichen Totenfalls eine leicht durchzuführende Methode, die das Bienenvolk oder die Milben selbst unbeeinflusst lässt. In der vorliegenden Untersuchung bestand eine enge Beziehung zwischen dem natürlichen Totenfall und der durch die anderen beiden Methoden ermittelten absoluten Milbenpopulation. Allerdings war die Methode nur für weiselrichtige Völker mit Brut verlässlich, und nur solange diese nicht nahe dem Zusammenbruch durch die Varroose waren. Zudem musste der Totenfall über mindestens eine Woche zusammengefasst werden.

***Varroa destructor* / *Apis mellifera* / Populationsgrösse / Beprobungsmethode / natürliche Mortalität**

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