

EFFECTS OF AGGREGATION AND SAMPLE SIZE ON VARROA MITE MONITORING: A SIMULATION APPROACH

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BACKGROUND

IPM is a valuable strategy for organic beekeeping: as organic relies on "soft treatments", it has to combine multiple interventions to keep varroa mites under economic damage thresholds. Monitoring varroa infestation to identify bee yards that are in need of treatment and to choose the treatment option is a fundamental part of IPM strategies.

Macroparasites are typically aggregated across their host populations, with most individuals harbouring relatively low numbers of parasites, while a few individuals harbour very many parasites (Shaw e Dobson 1995). It can be due to different exposure to the parasite (in case of varroa for robbing and drifting), but also in the absence of any heterogeneity in exposure, even small differences in susceptibility between hosts can rapidly produce non-random, aggregated distributions of parasites (Anderson and May 1978).

Parasite aggregation across hosts introduces biases in sample estimates of average infestation rates, in particular when sample sizes are small (Gregory e Woolhouse 1993).

Our aim was to (1) estimate varroa mite aggregation across bee colonies from field data and (2) create a realistic big dataset representing 10000 apiaries and simulate sampling from it with different sample sizes to evaluate sample size effect on estimate accuracy.

MATERIALS & METHODS

Estimation of varroa mite aggregation.

We measured adult bee infestation rate of individual colonies from 12 commercial bee yards in northern Italy by the means of sugar shaking method, sampling 300 bees from brood frames (Lee et al. 2010; Lee, Reuter, and Spivak 2010). Bee yards consistence ranged from 20 to 94 colonies (mean 47.9, sd 20.5). Apiaries infestation was measured between June, 9th and August, 5th.

We modeled varroa aggregation by the means of negative binomial distribution, a common approach in parasitology (Schmid and Robinson 1972).

For each apiary we estimated the distribution parameters (`fitdistr` routine, Venables and Ripley 2002): mean and k , the parameter for aggregation and fitted a linear regression model of their relationship.

Simulation of apiaries and sampling.

We simulated the infestation of 10000 apiaries composed of 100 colonies with mean infestation levels equally spaced from 0.5 to 10% and k parameter derived from the linear model previously fitted, also including the random error.

We randomly sampled (100 replicates) from each simulated apiary. Different scenarios were simulated, with sample size of 4, 8, 10, 12 and 14 colonies. Then we calculated: the rate of estimates higher than 2%, a commonly adopted treatment threshold (e.g. Currie and Gatten 2006), and related it (1) to actual mean infestation and (2) to the rate of colonies with infestation over 5%, proposed as the epidemic threshold for DWV (Sumpter and Martin 2004).

The analysis was performed using R statistical software (R Development Core Team 2013), for every simulation has been set a seed for the pseudo-random number generator in order to make the analysis fully reproducible (Peng 2011). The code is available from the authors upon request.

RESULTS & DISCUSSION

Varroa mite aggregation.

We estimated mean and k parameters for each apiary. Means ranged from 1.3 to 43.9%. The most apiary, with 43.9% mean infestation, was graphically spotted as an outlier and excluded from the analysis. Moreover such bee yard was out of our range of interest (it was the only bee yard with colonies collapsing for varroa infestation). Varroa resulted always aggregated across colonies ($k < 5$), in least infested apiaries it resulted strongly aggregated ($k < 1$).

We modeled the relationship between the two parameters with a linear regression model (table 1 and figure 1).

The strongest aggregation associated with low mean infestations is likely due to the limits of detection of sugar shaking method: at low means the zero results are not true zeros, but colonies infested under the limit of detection of the method.

	Estimate	Std. Error	t value	Pr(> t)
mean	0,21202	0,02086	10,16	3 E-007

Table1: linear model parameter of the relationship between mean and k

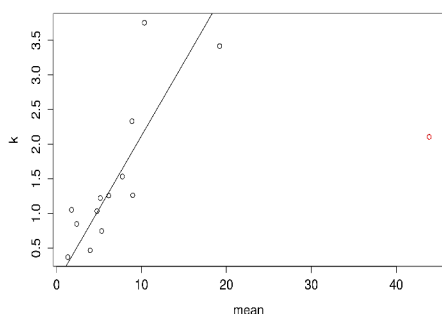


Figure 1: relationship between mean (mites/300 bees) and k parameters, linear regression line; in red the bee yard considered as outlier.

Simulation of apiaries and sampling.

1) Sample size effect. Probability of obtaining a sample mean greater than the treatment threshold (2%) is related to sample size. Not unexpectedly the higher sample size imply more accuracy (more probability to take the correct treatment decision). The gain in accuracy is not linear, so up to 8 samples the gain is consistent, while increasing sample size over 10 colonies there is only a marginal gain in accuracy (fig. 1 and 2). The choice of the right sample size requires an economical evaluation which is beyond the scope of this work.

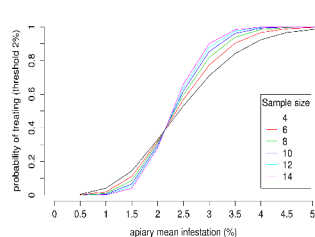


Fig 1: effect of sample size on the probability of obtaining a sample mean greater than the treatment threshold (2%) varying apary infestation level. Apary size was set at 100 colonies.

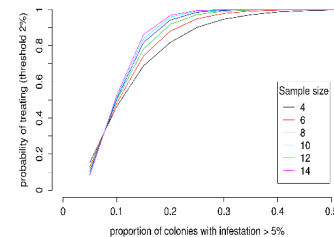


Fig 2: effect of sample size on the probability of obtaining a sample mean greater than the treatment threshold (2%) varying the proportion of colonies with infestation level greater than 5%. Apary size was set at 100 colonies.

2) Apary size effect. With a sample size of 8 colonies the size of the apary do not affect estimate accuracy. The accuracy is substantially the same with larger apiaries (fig.3).

3) Threshold effect. Such parameter is the one with most influence on treatment decisions (fig. 4). Higher thresholds imply less accuracy and less responsiveness when the threshold has actually been crossed. Sample size should set accordingly.

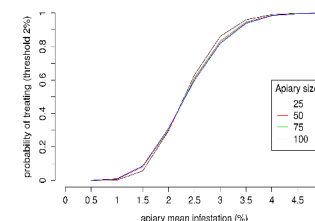


Fig 3: effect of apary size on the probability of obtaining a sample mean greater than the treatment threshold (2%) varying apary infestation level. Sample size was set at 100 colonies.

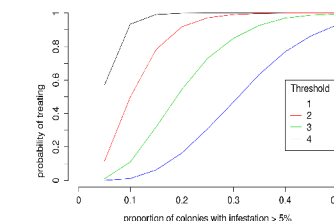


Fig 4: effect of threshold on the probability of taking a treatment decision varying the proportion of colonies with infestation level greater than 5%. Apary size was set at 100 colonies.

CONCLUSIONS

- Like other parasites, varroa mites are aggregate across host colonies.
- The least reliable sample size is 8 colonies chosen at random (like suggested by Lee, Reuter, and Spivak 2010). But from 8 to 10 colonies there is still an appreciable improvement of accuracy of the sample mean, so it is up to the operator to invest some more time to improve the accuracy of the estimate.
- Apary size has a very marginal influx on the accuracy of the estimate.
- Treatment thresholds have, obviously, a pivotal role in the probability of treating. Thresholds have to be set according to local and seasonal conditions (reviewed in Dietemann et al. 2013) and specific operation production goals. Setting appropriate thresholds is mainly an economical issue and goes beyond the scope of this study, but we advocate that threshold should be set together with appropriate sample size.
- Sugar shake results are reliable only in early-mid summer (unless extraordinary infestation). Natural mite drop count is suitable also in spring and it would be useful to study mite aggregation with the natural mite drop method along the active season.
- The effects of acaricide treatments on mite distribution still have to be explored. It is possible that the treatments alter aggregation, in such case the sample size should be accordingly modified.

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