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Scientific note

A scientific note on the ant pitfall for quantitative diagnosis of *Varroa destructor*

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The mite *Varroa destructor* is a global challenge for apiculture and accurate quantification crucial for adequate and timely pest management. However, foraging ants are regularly found in hives and may interfere with mite diagnosis. Here, we quantify for the first time the impact of ants. We expect lower mite numbers on bottom boards with foraging ants and that estimates of phoretic mites are ant independent.

From July to August 2007–2009, the experiments were conducted with 64 queenright honey bee colonies (predominantly *Apis mellifera carnica*, Table I). One apiary was used for 3 years, but each time, new colonies were monitored. All colonies (~11 frames of bees, 6–10 brood frames) were housed in Dadant hives (12 frames) with bottom board inserts for mite quantification (Imdorf et al. 2003) and placed in groups of four or five each on hive stands with four steel polders (50 cm aboveground). All colonies were treated in summer and fall using formic and oxalic acid (Imdorf et al. 2003). To quantify the impact of ants, we added traps (water-filled buckets [∅=20 cm]) to each of the four steel polders of the hive stands. At least once a month, water was refilled, and the surrounding vegetation was cut. The controls remained without traps. Quantifications were conducted weekly by removing the bottom board inserts and counting all mites and ants. In 2009, we also collected weekly 200 bee workers from the brood nests of 18 colonies for 8 weeks to evaluate the number of phoretic mites following Ritter and Ruttner (1980). For that purpose, nine colonies received traps (= treatments), and nine remained without (= controls) for 4 weeks; then, the groups were exchanged by relocating traps. Thus, we obtained from the same colonies mite infestation loads from both bottom board counts and phoretic mite estimates with or without ant traps. We also monitored all stands to investigate whether they were exposed to ant foraging. Ants were collected for taxonomic identification using morphometrics (Seifert 2007).

We performed Kruskal–Wallis and Mann–Whitney *U* post hoc tests to compare ant and mite numbers. To test whether there is a regression between ant and mite numbers, we constructed a Linear Mixed Model (LMM), with log-transformed *V. destructor* numbers as the dependent variable, log-transformed ant numbers as fixed effect and colony as random effect, resulting in normally distributed residuals:

$$Y_{ij} = \mu + \gamma_i + \beta(\alpha_{ij} - \overline{\alpha}) + \varepsilon_{ij}$$

where,

Y_{ij} Log10 (*V. destructor* numbers) in colony i at time j
μ Overall mean

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Table I. *V. destructor* mites and ants collected weekly from bottom boards of treatment and control hives at two apiaries (B = Bellechasse, W = Witzwil) in 2007, and one apiary in 2008–2009.

Apiary	Year	Group	Colonies	Mites		Ants		
			N	Numbers	*P* value	Numbers	Species	*P* value
W	2007	T	9	12 [0; 97]	$P<0.001$	0 [0; 7]	Ln, Ff	$P<0.001$
		C	8	1 [0; 40]		11 [0; 80]	Ln, Ff	
B	2007	T	5	43 [5; 255]	$P<0.001$	0 [0; 4]	Lb, Ln	$P<0.001$
		C	5	10 [0; 80]		2 [0; 26]	Lb, Ln	
W	2008	T	9	30 [4; 196]	$P<0.001$	0 [0; 0]		$P<0.001$
		C	10	4.5 [0; 269]		0 [0; 28]	Ln, Ff	
W	2009	T	9	43 [2; 252]	$P<0.001$	2.5 [2; 3]	Ln, Ff	$P<0.001$
		C	9	10 [1; 277]		5 [1; 36]	Ln, Ff	

Numbers are shown as medians [1st and 4th quartiles]. The identified ant species (Lb = *Lasius brunneus*, Ln = *Lasius niger*, Ff = *Formica fusca*) and the results of the Kruskal–Wallis tests between treatments (T) and controls (C) are shown

γ_i Random effect from colony i, normally distributed $\gamma_i \sim N(0, \sigma^2{}_\gamma)$

β Regression coefficient for the variable log10 (ant numbers)

a_{ij} Log10 (ant numbers) in colony i at time j

$\bar{a}$ The arithmetic mean of all a_{ij}

ε_{ij} Random error, normally distributed $\varepsilon_{ij} \sim N(0, \sigma^2{}_\varepsilon)$.

Moreover, we calculated the arithmetic means for colonies with both estimates of phoretic mites and bottom board counts under treatment and control conditions and then calculated Spearman's rank correlations to test for correlations between. All analyses were performed using Systat 13® and SPSS statistics 17®.

All hive stands were exposed to ant foraging. Three different species were found (Table I). All observed ants (N=2,006) were alive and occasionally observed carrying dead or alive mites, but never carrying dead bees or bee brood. In 2009, one colony died leaving N=17 for the estimates of phoretic mites. In each year, we found significant differences between treatments and controls. The LMM estimated model parameters were: (μ, $\sigma^2{}_\gamma$, β, $\sigma^2{}_\varepsilon$)=(1.3321, 0.1009, −0.6928, 0.1877) with $P<0.001$. The numeric equation was:

$$Y = 1.3321 - 0.6928\left(a_{ij} - \bar{a}\right).$$

No significant differences were found for the number of phoretic mites between controls (2.247 [1.508; 12.5]) and treatments (1.970 [1.163; 14.483]; Mann–Whitney U test, P=0.539). In the controls, we found no significant correlation between phoretic mites and natural mite fall estimates (N=17, r_s=0.097, P=0.937), but a significant positive one was found in the treatments (N=17, r_s=0.7574, $P<0.001$).

With the exception of *Lasius brunneus*, the collected ant species have repeatedly been reported from honey bee colonies (Burrill 1926 among others). The 22 ants found in treatment colonies were only detected at the initial surveys suggesting that they were trapped when installing the buckets. The controls had consistently lower mite numbers on the bottom boards compared to the treatments, showing that ant foraging can interfere with mite counts, thereby bearing the risk of inadequate pest management decisions. However, the estimates of phoretic mites were not different between treatments and controls, indicating that this constitutes an ant-independent approach. Indeed, there was no significant association between estimates of phoretic mite and natural mite fall in the controls, whereas a significant positive correlation was found in the treatments. We recommend to implement adequate ant traps into Good Apicultural Practice, whenever bottom board counts are used for *V. destructor* diagnosis.

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Note scientifique sur l'influence des fourmis pour le diagnostic quantitatif de *Varroa destructor*

Eine wissenschaftliche Notiz zum Einfluss von Ameisen auf die quantitative Diagnose von *Varroa destructor*

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