Supplementary information

Interaction between *Varroa destructor* and imidacloprid reduces flight capacity of honey bees

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Appendix S1: Justification of the imidacloprid treatment

We provided imidacloprid in sugar water to the colonies because bees are primarily exposed via nectar and pollen. Pollen feeding is very difficult to standardise. While flying, bees mostly feed a sugar diet [1] and metabolise relatively high amounts of sugars [2]. The supplied sugar is quickly distributed through the colony as is illustrated by [3]. In [3], bees (n=6) were fed with labelled sugar (radioactive phosphor) and their distribution of this labelled sugar via trophalaxis was monitored within the colony (24500 bees). Within 4 hours, 62% of all foragers had ingested some of this labelled sugar, after 27 hours 76% of all foragers had ingested some of this labelled sugar and in 43-60% of all other bees. Nurse bees had less labelled sugar and foragers relatively more.

We estimated that the exposed bees had a weekly sub-lethal exposure of 11% of the acute oral LD_{50} (=3.7 ng/bee). This is thought to be a forager lifetime exposure as bees forage on average seven to eight days with a minimum of about five and a maximum of nineteen days [4,5]. To estimate the forager's exposure, we calculated the colony size by monthly photographing the topside of the hive. 90±15% of the top side was covered with bees between July and September, corresponding with approximately 15000 bees [6]. Assuming that ⅓ of the bees in the colonies were foragers that consumed ½ of the fed sugar water each week, each forager would be exposed to 0.39 ng imidacloprid per week (half of 3945 ng imidacloprid in 660 ml, divided over 5000 foragers per colony). The remaining bees and larvae were assumed to consume the other half of the sugar water.

In relation to the potential field exposure, we estimated that the exposure of the pollen foragers we collected for testing was 1.12 to 1.68× a field-realistic concentration. Namely, 660 ml of sugar water per week contained 409 g of sugar, supplying each forager with 41 mg of sugar per week (5000 foragers dividing half of the sugar), while an average pollen forager normally consumes 73-110 mg sugar per week (225-900 mg for a nectar forager) [7]. Our supplied sugar therefore comprises of 37-56% of the sugar diet of an average pollen forager. To be exposed to field conditions, pollen foragers should forage for 37-56% of their time on a field supplying nectar with the concentration we provided (5.98±0.22 ng/ml a.i. imidacloprid). However, the concentration of imidacloprid in nectar of sunflower or canola was found to be 1.9 ppb (ng/ml) [8] and the concentration of clothianidin (another neonicotinoid) in nectar of canola was found to be 2.24 ppb (ng/ml) [9]. To gain a similar exposure as the concentration we fed the bees, pollen foragers should spend 112-168% of their time collecting food from fields with on average a concentration of 2 ng/ml. As bees can only spend maximally 100% of

their time foraging on a field, this means that the exposure of the pollen foragers is a worst case field-realistic concentration.

Twice a week samples were taken of the bulk sugar water containing imidacloprid. The research institute RIKILT Wageningen UR, The Netherlands, determined the imidacloprid concentrations of the samples by the QuEChERS method (acetonitrile extraction and LC-MS*MS [10]).

References

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Appendix S2: Supplementary movie

This movie illustrates how a honey bee was tethered on a flight mill:

https://www.youtube.com/watch?v=XnHGJBNtxK8

Appendix S3: Additional results

Table S1. Results of the linear mixed models for the effects of the stressors *Varroa destructor* and a field-realistic, chronic sub-lethal dose of the neonicotinoid insecticide imidacloprid including their interaction on the flight time, average flight speed and maximum flight speed of forager honey bees tethered on a flight mill. For each of these dependent variables, the model numbers refer to the different arrows in Figure S1. For model 5, we took the residuals from model 2 as the dependent variable. For each factor in the model, the F- and P-value are given. Some models include a significant covariate (BM = body mass, WL = wing length) from which we report the parameter estimate and the standard error of the estimate. For the random variable Colony, the Wald-statistic and P-value are given. For each model, we give the applied method of estimation (ML= Maximum Likelihood), whether we log-transformed the dependent variable (Log), the sample size (N), the value of the AICc and the Repeated Covariance Type (DIAG = Diagonal, UN=Unstructured).

This caption belongs to the Table on page 6, 7 and 8

Table S1. Continued

Model nr.		**1**	**2**	**3**	**4**	**5**
Independent variables	Statistics	Flight time (min)	Flight time (min)	Flight time (min)	Flight time (min)	Residuals of model 2**
1M-2M	F	66.10	59.15	59.70	63.81	-
	P	<0.001	<0.001	<0.001	<0.001	-
Varroa	F	4.17	-	-	4.21	-
	P	0.047	-	-	0.047	-
Imidacloprid	F	1.31	-	-	1.37	-
	P	0.26	-	-	0.25	-
Varroa x Imidacloprid	F	4.29	-	-	5.76	-
	P	0.04	-	-	0.02	-
Body mass (mg)	F	-	2.95	-	-	-
	P	-	0.09	-	-	-
Wing length (mm)	F	-	-	1.26	2.25	-
	P	-	-	0.27	0.14	-
Colony	Wald Z	*	*	0.76	*	-
	P	*	*	0.45	*	-
Covariate estimate		-	-0.007	0.007	0.009	-
Std. error		-	0.004	0.006	0.006	-
Estimation method		ML	ML	ML	ML	-
Transformation		Log	Log	Log	Log	-
N		54	54	54	54	-
AICc		24.99	25.65	26.76	25.80	-
Repeated Covariance Type		DIAG	DIAG	DIAG	DIAG	-

- This variable was not tested in the model

* Although this variable was redundant, the test statistic and confidence interval could not be computed, we kept the variable in the model

** Model 2 showed no effect of Body mass on Flight time, therefore model 5 becomes redundant

Table S1. Continued

Model nr.		**1**	**2**	**3**	**4**	**5**
Independent variables	Statistics	Average speed (m/s)	Average speed (m/s)	Average speed (m/s)	Average speed (m/s)	Residuals of model 2**
1M-2M	F	29.05	28.26	28.40	27.70	-
	P	<0.001	<0.001	<0.001	<0.001	-
Varroa	F	0.001	-	-	0.002	-
	P	0.97	-	-	0.97	-
Imidacloprid	F	0.09	-	-	0.13	-
	P	0.76	-	-	0.72	-
Varroa x Imidacloprid	F	0.07	-	-	0.01	-
	P	0.79	-	-	0.92	-
Body mass (mg)	F	-	1.07	-	-	-
	P	-	0.31	-	-	-
Wing length (mm)	F	-	-	0.80	0.78	-
	P	-	-	0.38	0.38	-
Colony	Wald Z	*	*	*	*	-
	P	*	*	*	*	-
Covariate estimate		-	-0.004	0.005	0.005	-
Std. error		-	0.003	0.005	0.006	-
Estimation method		ML	ML	ML	ML	-
Transformation		-	-	-	-	-
N		54	54	54	54	-
AICc		-5.42	-11.92	-11.71	-3.16	-
Repeated Covariance Type		UN	UN	UN	UN	-

- This variable was not tested in the model

* Although this variable was redundant, the test statistic and confidence interval could not be computed, we kept the variable in the model

** Model 2 showed no effect of Body mass on Flight time, therefore model 5 becomes redundant

Table S1. Continued

Model nr.		**1**	**2**	**3**	**4**	**5**
Independent variables	Statistics	Maximum speed (m/s)	Maximum speed (m/s)	Maximum speed (m/s)	Maximum speed (m/s)	Residuals of model 2**
1M-2M	F	10.59	10.27	9.78	9.54	-
	P	0.003	0.003	0.004	0.004	-
Varroa	F	1.32	-	-	1.20	-
	P	0.26	-	-	0.28	-
Imidacloprid	F	2.17	-	-	2.45	-
	P	0.15	-	-	0.13	-
Varroa x Imidacloprid	F	0.002	-	-	0.04	-
	P	0.96	-	-	0.84	-
Body mass (mg)	F	-	1.04	-	-	-
	P	-	0.32	-	-	-
Wing length (mm)	F	-	-	1.77	1.86	-
	P	-	-	0.19	0.18	-
Colony	Wald Z	*	*	*	*	-
	P	*	*	*	*	-
Covariate estimate		-	-0.004	0.008	0.008	-
Std. error		-	0.004	0.006	0.006	-
Estimation method		ML	ML	ML	ML	-
Transformation		-	-	-	-	-
N		54	54	54	54	-
AICc		13.35	9.54	8.89	14.63	-
Repeated Covariance Type		UN	UN	UN	UN	-

- This variable was not tested in the model

* Although this variable was redundant, the test statistic and confidence interval could not be computed, we kept the variable in the model

** Model 2 showed no effect of Body mass on Flight time, therefore model 5 becomes redundant

Table S2. Results of the correlations for the different models in Figure S2 (model numbers refer to the different arrows). For each correlation, the Pearson correlation coefficient r, P-value and the sample size N are given.

Model nr.	**8**	**9**	**10**	**11**	**12**	**13**
r	-0.059	-0.109	0.061	0.969	0.694	0.576
P	0.747	0.553	0.741	<0.001	<0.001	0.001
N	32	32	32	32	32	32

Table S3. Results of the two-way ANOVA for the effects of the stressors *Varroa destructor*, a field-realistic, chronic sub-lethal dose of the neonicotinoid insecticide imidacloprid and their interaction on the number of *V. destructor* mites per gram bees on colony level (log-transformed (+0.01)), and weighted for the final number of bees tested per colony. In total, 258 attempts were made to fly bees from 25 colonies (6-7 colonies per group). Tests were separately done for bees that successfully flew in the flight mill (32 bees, ranging from 0-7 bees per colony) or did not (226 bees, ranging from 0-20 bees per colony). For each factor in the model, the F- and P-value are given.

Independent variables	Statistics	Bees that did not fly	Bees that did fly
Varroa	F	74.65	78.07
	P	<0.001	<0.001
Imidacloprid	F	0.01	6.49
	P	0.91	0.03
Varroa x Imidacloprid	F	0.16	6.58
	P	0.69	0.02

Figure S1. The relationships that were tested in linear mixed models explaining flight time, speed and maximum speeds of forager honey bees tethered on a flight mill. The numbers of the arrows refer to the models in Table S1. For example, model 4 includes the effects of the treatments (*Varroa destructor*, imidacloprid and the interaction) and wing length.

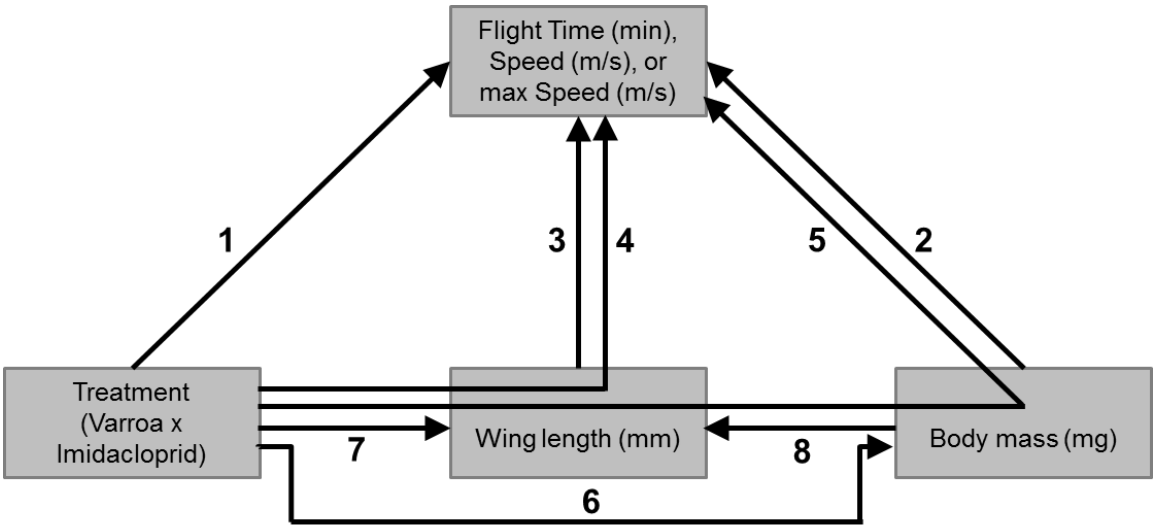


Figure S2. The relationships that were tested as correlations between body mass, abdomen mass, thorax mass and wing length. The numbers of the arrows refer to the models in Table S2. For example, model 8 is the correlation between body mass and wing length.

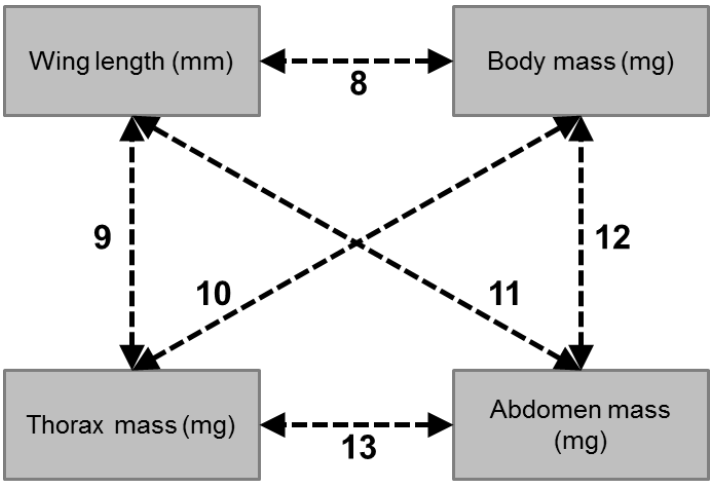
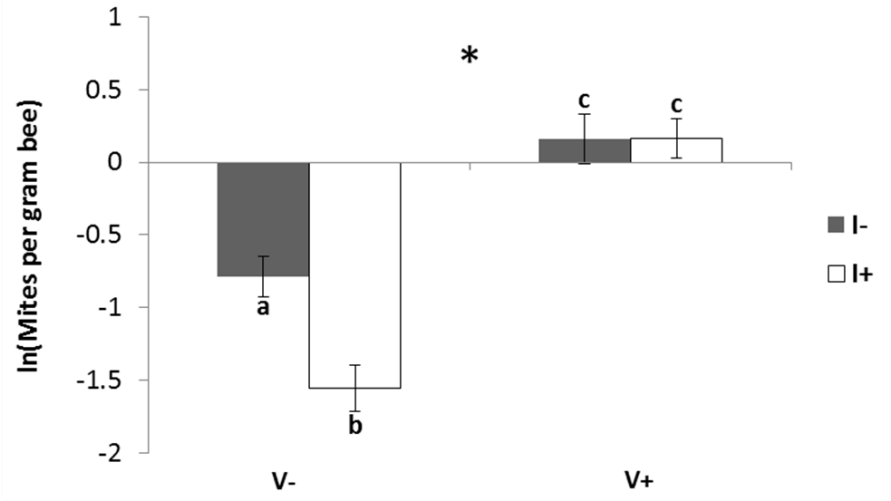


Figure S3. Mean number of mites per gram bees (log-transformed (+0.01)) due to the stressors *Varroa destructor* infestation (V- refers to colonies that were treated against *V. destructor*, whereas the colonies V+ were not treated) and to different concentrations of a field-realistic, chronic sub-lethal dose of the neonicotinoid insecticide imidacloprid (I- refers to colonies with no exposure, whereas colonies I+ were exposed). Error bars indicate the standard error of the mean. In total, 258 attempts were made to fly bees in the flight mill. Tests were separately done for (a) bees that successfully flew in the flight mill (32 bees) and for (b) bees that did not (226 bees). The letters give the significant differences between the treatment combinations based on two separate two-way ANOVA (Table S3).

(a)



(b)