Supplementary Information for

Field-level clothianidin exposure affects bumblebees but generally not their pathogens

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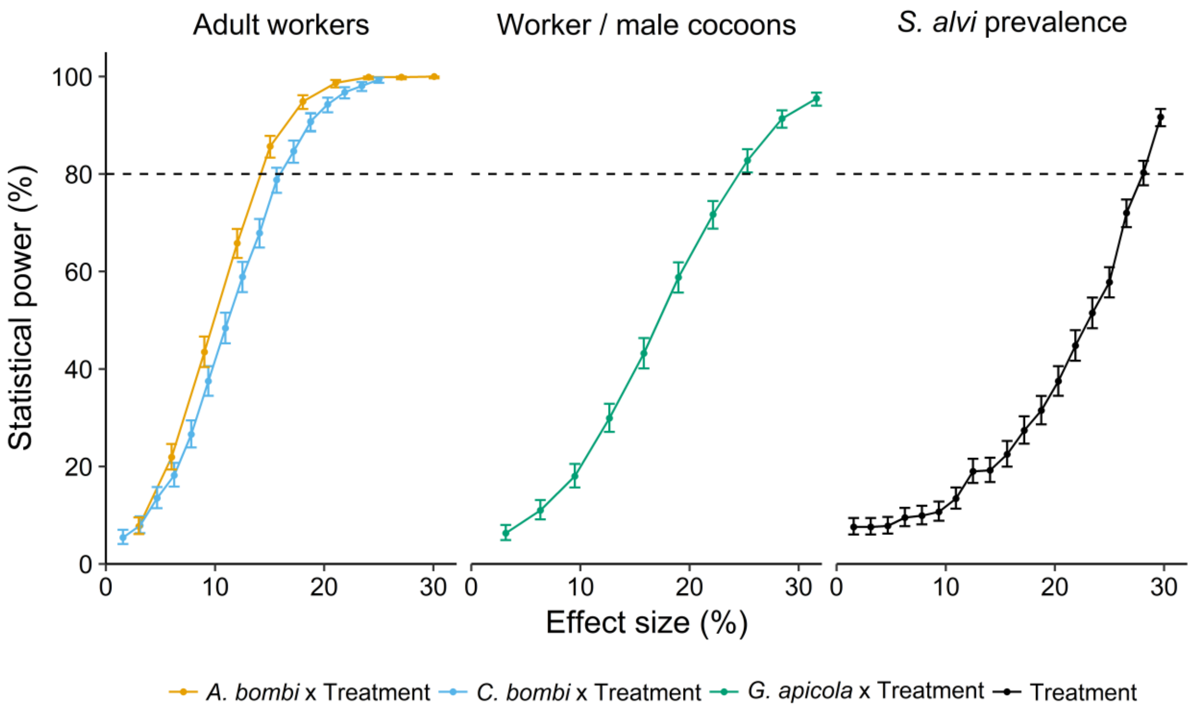
This PDF file includes:

Supplementary Figure 1

Supplementary Tables 1-4

Supplementary References

Supplementary Figures



Supplementary Figure 1 | Statistical power. Power in relation to effect size for treatment effects or interactive effects between treatment and microorganism abundance where $0.05<P<0.1$. For interactive effects on the numbers of adult workers and worker/male cocoons, effect size represents the effect of a $\log_{10}$ unit increase in microorganism abundance expressed as a percentage of the estimated value of a control colony with average microorganism $\log_{10}$ abundance. For *S. alvi* prevalence effect size represents an increase in the number of infected colonies illustrated as a percentage of all colonies. The dashed line indicates a power of 80%.

Supplementary Tables

Supplementary Table 1 | Microorganism prevalence. The prevalences of detected microbiota in adult worker bees were related to clothianidin seed treatment using two different kinds of tests: tests of equal proportions for straight comparisons of the proportions of infected colonies between treatments and analyses of variance (ANOVA) based on the number of infected colonies per field to control for non-independence of colonies placed by the same field.

Response	**Predictor**	**Test**	**Estimate** [c]	χ^2_1	***P*** [d]
Acute bee paralysis virus	Treatment	Equal proportions [a]	-9.4%	0.87	0.352
Slow bee paralysis virus	Treatment	Equal proportions [a]	6.3%	1.84	0.668
Sacbrood virus	Treatment	LRT on GLMM [b]	12.2%	1.74	0.189
Apicystis bombi	Treatment	LRT on GLMM [b]	-14.7%	0.71	0.400
Crithidia bombi	Treatment	LRT on GLMM [b]	-20.8%	4.33	**0.037**
Nosema bombi	Treatment	Equal proportions [a]	-9.4%	0.87	0.352
Gilliamella apicola	Treatment	Equal proportions [a]	0%	0	1
Snodgrassella alvi	Treatment	LRT on GLMM [b]	23.8%	3.63	0.057

[a] Two-sided test of equal proportions using the prop.test function in R

[b] Likelihood ratio test on a generalized linear mixed effects model with field identity as random effect.

[c] Effect sizes are expressed as absolute differences between treatments in the percentage of infected colonies.

[d] *P*-values < 0.05 are highlighted in bold

Supplementary Table 2 | Microorganism abundance. Log_{10} abundance of prevalent microorganisms in worker bees of infected colonies (i.e. excluding colonies in which the target organism was not detected) in relation to clothianidin seed treatment [a].

Response	Predictor	Estimate ($\log_{10}$ units)	Estimate (%) [a]	χ^2_1	P [b]	N Fields	N Colonies
A. bombi	Treatment	1.09	24.5	2.39	0.122	15	40
C. bombi	Treatment	- 0.68	- 11.3	2.70	0.100	16	53
G. apicola	Treatment	0.28	5.39	1.39	0.239	16	58
S. alvi	Treatment	0.05	1.10	0.13	0.720	16	50

[a] Effect sizes in % were calculated in reference to the control group

[b] *P*-values were calculated by likelihood ratio tests with 1 degree of freedom on linear mixed effects models containing field identity and field pair identity as random factors.

Supplementary Table 3 | Primers. Forward (F) and reverse (R) primers used in RNA/DNA assays with a literature reference to previous usage.

Target	**Primers**	**Sequence '5-'3**
Acute bee paralysis virus[1]	ABPV-F6548 (F) KIABPV-B6707 (R)	TCATACCTGCCGATCAAG CTGAATAATACTGTGCGTATC
Black queen cell virus[1]	BQCV-qF7893 (F) BQCV-qB8150 (R)	AGTGGCGGAGATGTATGC GGAGGTGAAGTGGCTATATC
Chronic bee paralysis virus[1]	CBPV1-qF1818 (F) CBPV1-qB2077 (R)	CAACCTGCCTCAACACAG AATCTGGCAAGGTTGACTGG
Deformed wing virus[1]	DWV-F8668 (F) DWV-B8757 (R)	TTCATTAAAGCCACCTGGAACATC TTTCCTCATTAACTGTGTCG
Lake Sinai Virus type-1[1]	qLSV1-F2569 (F) qLSV1-R2743 (R)	AGAGGTTGCACGGCAGCATG GGGACGCAGCACGATGCTCA
Lake Sinai virus type-2[1]	qLSV2-F1722 (F) qLSV2-R1947 (R)	CGTGCTGAGGCCACGGTTGT GCGGTGTCGATCTCGCGGAC
RNA250[2]	RNA250 (F) RNA250 (R)	TGGTGCCTGGGCGGTAAAG TGCGGGGACTCACTGGCTG
Bt-RPL23[3]	Bt-RPL23 (F) Bt-RPL23 (R)	GGGAAAACCTGAACTTAGGAAAA ACCCTTTCATTTCTCCCTTGTTA
Slow bee paralysis virus[4]	SBPV-F3177 (F) SBPV-B3363 (R)	GCGCTTTAGTTCAATTGCC ATTATAGGACGTGAAAATATAC
Sacbrood virus[1]	SBV-qF3164 (F) SBV-qB3461 (R)	GCTCTAACCTCGCATCAAC TTGGAACTACGCATTCTCTG
Apis mellifera filamentous virus[5]	AmFV-BroN (F) AmFV-BroN (R)	TTATTAACACCGCAGGCTTC CATGGTGGCCAAGTCTTGCT
Gregarine spp.[6, b]	Gregarine (F) Gregarine (R)	CCAGCATGGAATAACATGTAAGG GACAGCTTCCAATCTCTAGTCG
Crithidia bombi [a,b]	Cbombi-GADH-F2 (F) Cbombi-GADH-R2 (R)	CAAGAGCTCGCCGGGT GGACGCGTTCGACACC
Nosema apis [7]	Napis-qF3 (F) NosUniv-qR3 (R)	TAGTATATTTGAATATTTGTTTACAATGG CGCTATGATCGCTTGCC
Nosema ceranae [a, b]	Nceranae-qF3 (F) NosUniv-qR3 (R)	GTATGTTTGAATAATTATTTATTTTATTG CGCTATGATCGCTTGCC
Nosema bombi [a, b]	Nbombi-qF3 (F) NosUniv-qR3 (R)	TAGTATGTTTGAATATTTATTTATTACGA CGCTATGATCGCTTGCC
Gilliamella apicola [a,b]	Gilliam 16S (F) Gilliam 16S (R)	GTAACATGAGTGCTTGCACT CGCATGGCCCGAAGG
Snodgrassella alvi [a,b]	Snodgras 16S (F) Snodgras 16S (R)	ACGGAGAGCTTGCTCTC AAATAACGCGAGGTCTTTCGA

[a] The primers were first used in this study.

[b] Identity of target organism confirmed by bidirectional Sanger sequencing of selected PCR products. In all cases, the PCR product sequences matched 100% with the intended target.

Supplementary Table 4 | Data analysis excluding the Biscaya sprayed field. Tests that resulted in a change of the level of significance (based on α = 0.05) if the field that was sprayed with Biscaya (containing thiacloprid) was removed from the data analysis.

Response	**Predictor**	**Sample**	**Estimate**	χ^2_1	***P*** [a]
Crithidia bombi prevalence	Treatment	All colonies	-20.8%	4.33	**0.037**
Crithidia bombi prevalence	Treatment	Excluding Biscaya sprayed field	-20.1%	3.63	0.057
Worker/male cocoons	*Apicystis bombi*	All colonies	-8.946	3.70	0.054
Worker/male cocoons	*Apicystis bombi*	Excluding Biscaya sprayed field	-14.174	5.08	**0.024**

[a] *P*-values are based on likelihood ratio test on a generalized linear mixed effects model with field identity as random effect.

Supplementary References

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