

## Supplementary Material

### Title

Neonicotinoid Clothianidin reduces honey bee immune response and contributes to *Varroa* mite proliferation

### Authors

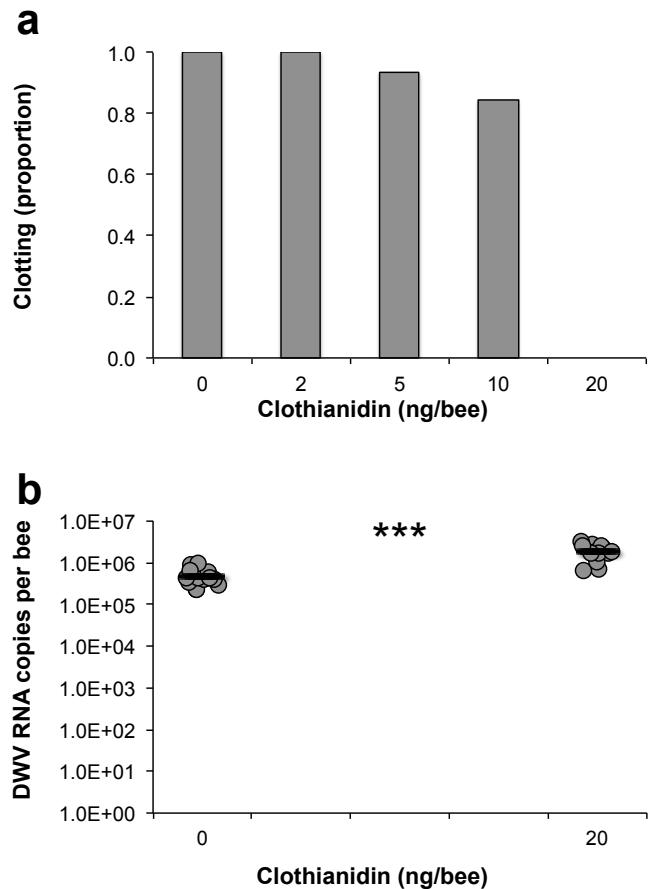
Desiderato Annoscia, Gennaro Di Prisco, Andrea Becchimanzi, Emilio Caprio, Davide Frizzera, Alberto Liguadoca, Francesco Nazzi & Francesco Pennacchio

### Corresponding authors

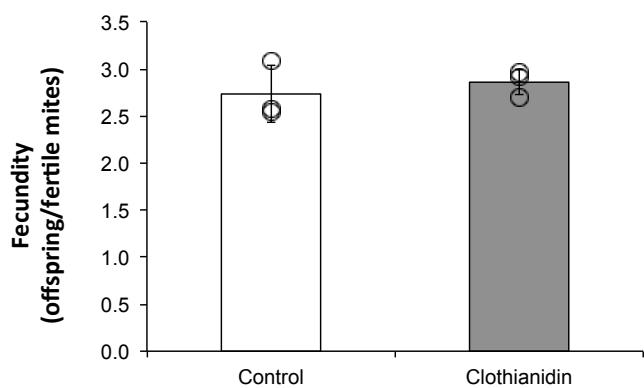
Francesco Nazzi, [francesco.nazzi@uniud.it](mailto:francesco.nazzi@uniud.it)

Francesco Pennacchio, [f.pennacchio@unina.it](mailto:f.pennacchio@unina.it)

Supplementary figures



Supplementary Figure 1. **Effect of Clothianidin treatment on clotting and DWV infection level in adult bees.** **a** Clotting (Spearman's rho=-0.975, n=5, P<0.05). **b** DWV infection level (Mann-Whitney U test: n<sub>1</sub>=15, n<sub>2</sub>=15, U=4, P<0.001). Three replicates, of five honey bees each, were considered for each experimental condition; the horizontal bars in the scatter-jittered plot represent the sample average.



**Supplementary Figure 2. Fecundity (offspring per fertile female) of *Varroa* mites feeding on pupae treated or not with Clothianidin.** The experiment was run in triplicate, for a total of 78 ( $n=25+17+36$  biologically independent mites) and 68 ( $n=23+14+31$  biologically independent mites) individuals for Clothianidin treated and untreated controls, respectively; the offspring per fertile female in each replicate along with the average fecundity and relative standard deviation are reported.

## Supplementary tables

Supplementary Table 1. Primers used for qRT-PCR analysis of DWV and immune genes.

Transcript	Sequence
DWV	F: GCGCTTAGTGGAGGAAATGAA R: GCACCTACCGCGATGTAAATCTG
Dorsal 1A	F: ACAGGCAGAAGCTGAGAAC R: TTGCCATCGGATACAAGGAT
Amel\102	F: CAACTCCAGAATTGGAAATAGCA R: TTTGCAATAGGAAAAGCAGTTG
$\beta$ -actin	F: GATTGTATGCCAACACTGTCCTT R: TTGCATTCTATCTGCGATTCCA
rps5	F: AATTATTGGTCGCTGGAATTG R: TAACGTCCAGCAGAATGTGGTA