Supplementary Figures:

Supplementary Figs 1 and 2, and pictures of uncropped gels

Acute Thiamethoxam toxicity in honeybees is not enhanced by common fungicide and herbicide and lacks stress-induced changes in mRNA splicing

Pâmela Decio^{1*}, Pinar Ustaoglu^{2*}†, Thaisa C. Roat¹, Osmar Malaspina¹, Jean-Marc Devaud³, Reinhard Stöger⁴, ⁵ and Matthias Soller², ⁵

¹Universidade Estadual Paulista (UNESP), Instituto de Biociências, Centro de Estudos de Insetos Sociais, Rio Claro, São Paulo, Brazil.

²School of Biosciences, College of Life and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, United Kingdom

³Research Center on Animal Cognition, Center for Integrative Biology, Toulouse University, CNRS, UPS, Toulouse, France.

⁴School of Biosciences, University of Nottingham, LE12 5RD, United Kingdom

Running title: Neonicotinoid toxicity is not enhanced by common fungicide and herbicide

Key Words: Neonicotinoid, carbendazim, glyphosate, *Apis mellifera*, alternative splicing, *Dscam*, ELAV, RNA binding proteins

⁵Corresponding authors: <u>m.soller@bham.ac.uk</u>, <u>reinhard.stoger@nottingham.ac.uk</u>

* equal contributing authors

Present address: † MRC Centre for Molecular Bacteriology and Infection, and Department of Life Sciences, Imperial College London, Ground Floor, Flowers Building, South Kensington Campus, London SW7 2AZ, UK

Suppl Figure 1



Supplemental Figure 1: Picture of a honey bee. The red arrow points towards the sitre of injection.



Supplemental Figure 2: Denaturing polyacrylamide gel showing the splicing pattern of Dscam exon 4 isoform variables after digestion with restricition enzymes 5 h and 10 h after injection of Thiamethoxam (1 μ M) or the combination of Thiamethoxam, Carbendazim and Glyphosate (T+C+G, 1 μ M/2 mM/47 mM). The undigested PCR is shown at the bottom. Ma: DNA marker

Fig 2 C



Fig 2 D gel used for quantification



Fig 2 E



gel in Fig 3 AL AND THE OF 1.88 м undigested -280 м 4 . ---------10.00 Ann and \$100 ---------------... he for he will be -1





Control to Control Date Control Contro Ма

Control to Control Con Ма

undigested long run







Supplemental Figure 2 undigested

Supplemental Figure 2 digested