

Supplementary Information

Novel fungicide and neonicotinoid insecticide impair flight behavior in pollen foraging honey bees, *Apis mellifera*

Anna Keodara¹, Lukas Jeker², Lars Straub^{3,4,5}, Daniela Gossar², Jan Müller⁶, Verena Christen^{1,*}

¹ University of Applied Sciences and Arts Northwestern Switzerland, School of Life Sciences, Muttenz, Switzerland

² Agroscope, Swiss Bee Research Center, Schwarzenburgstrasse 161, Bern, Switzerland

³ Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland

⁴ Faculty of Science, Energy and Environment, King Mongkut's University of Technology North Bangkok, Rayong Campus, Rayong, Thailand

⁵ Centre for Ecology, Evolution, and Behaviour, Department of Biological Sciences, Royal Holloway University of London, Egham, United Kingdom

⁶ Federal Office of Information Technology, Systems and Telecommunication, Bern, Switzerland

Tables:

S1: Chemical analysis data of sucrose-pyraclostrobin solutions.

S2: Exposure concentration.

S3: Summarized flight data for analysis of gene expression.

S4: Summarized flight data for longevity.

S5: Primer sequences and qPCR efficiency of analyzed transcripts.

S6: RFID median flight duration of foragers of all treatment groups.

S7: Out time data of foragers of all treatment groups.

S8: Multiple comparisons test of transcript level linked to energy metabolism in foragers performing homing flight.

S9: Multiple comparisons test of transcript level linked to endocrine systems in foragers performing homing flight test.

S10: Multiple comparisons test of transcript level linked to energy metabolism in foragers **without** homing flight.

S11: Multiple comparisons test of transcript level linked to endocrine systems in foragers **without** homing flight.

Figures:

S1: Graphical representation of the RFID experiment.

S2: Weather data of the three experimental days.

S3: RFID system installed at the entrance of a honeybee colony.

S4: CT-values of reference transcript *rps5* in the brain of foragers of the four treatment groups.

S5: Control vs. acetone in terms of survival, return rate and homing flight duration of pollen foragers in RFID test.

S6: Flight duration of foragers of the three different colonies.

S7: Out time in hours of foragers of all treatment groups.

S8: Expression level of *cox5a*, *cox5b* and *cox17* of foragers performing homing flight.

S9: Expression level of *buffy*, *hbg-3* and *vitellogenin* of foragers performing homing flight.

S10: Expression level of *buffy*, *hbg-3* and *vitellogenin* of foragers **without** homing flight.

Table S1: Chemical analysis data of sucrose-pyraclostrobin solutions.

Date of analysis	Nominal concentration	Measured concentration	% of nominal concentration	mean concentration
07.07.2021	2.65 µg/bee	2.43 µg/bee	92	2.43 µg/bee
	2.65 µg/bee	2.42 µg/bee	91	
	100 µg/bee	5.85 µg/bee	59	5.98 µg/bee
	100 µg/bee	6.10 µg/bee	61	

Table S2: Exposure concentration

Pesticide	Exposure Concentration	Acetone Concentration	LD ₅₀ (oral acute)
Pyraclostrobin	2.43 and 5.98 mg/bee	5%*	>110 mg/bee ^a
Thiamethoxam	1 ng/bee	5%*	5 ng/bee ^b

*Pyraclostrobin has a low solubility in water (PPDB, 2021b) and was therefore dissolved in 5% acetone (OECD Guideline, 2017). For uniform handling, thiamethoxam was also dissolved in 5% acetone.

^a PPDB, 2021b

^bFood and Authority, 2013; PPDB, 2021a

Table S3: Summarized flight data for analysis of gene expression.

Homing flight duration and gene expression analysis					
Treatment group	C	SC	PS 2.43	PS 5.98	TMX
N° of bees chipped for gene expression	60	60	60	60	60
N° of not started bees	5	7	2	3	7
N° of bees started	55	53	58	57	53
N° of not returned bees	10	18	22	22	15
N° of returned bees within 24h	45	38	36	35	38
N° of returned bees after 2h (not sampled for GE analysis, continue monitoring for longevity)	9	9	14	17	4
N° of returned bees <u>within 2h</u> (measured homing flight duration and sampled for GE analysis)	36	29	22	18	34

Flight data from RFID experiment for gene expression analysis. Definition: Not started bees: bees were not able to perform the homing flight after the treatment process. Legends: C = 40% sucrose control, SC = acetone solvent control, TMX = 1ng thiamethoxam/ bee, PS 2.43 = 2.43 µg/bee, PS 2 = 5.98 µg/bee, GE = gene expression.

Table S4: Summarized flight data for longevity.

Longevity assessment (out time and survival time)					
Treatment group	C	SC	PS 2.43	PS 5.98	TMX
N° of bees chipped for longevity	60	60	60	60	60
N° of not started bees	4	2	4	9	7
N° of started bees	56	58	56	59	53
N° of not returned bees	10	16	21	21	18
N° of returned bees	46	42	35	30	35
N° of bees from part "gene expression" that returned after 2h and thus continued to be monitored and included in longevity assessment	9	9	14	17	4
N° of total bees (L+GE) monitored for longevity	55	51	49	47	39
N° of invalid flights	9	13	9	10	6
total N° of bees out-time	46	38	40	37	33

Flight data from RFID experiment for longevity assessment. Definition: Not started bees: bees were not able to perform the homing flight after the treatment process. Invalid flights: e.g. less than 2 min out-hive time. Legends: C = 40% sucrose control, SC = acetone solvent control, TMX = 1ng thiamethoxam/ bee, PS 2.43 = 2.43 µg/bee, PS 2 = 5.98 µg/bee, L = Longevity, GE = gene expression.

Table S5: Primer sequences and qPCR efficiency of analyzed transcripts.

Function	Primer	Sequence (5' → 3')	Efficiency	Source
Housekeeping gene	<i>ribosomal protein s5 (rps5)</i>	Forward AATTATTTGGTCGCTGGAATTG Reverse TAACGTCCAGCAGAATGTGGTA	1.94	(Evans et al., 2006)
Oxidative phosphorylation	<i>cytochrome c oxidase subunit 5a (cox5a)</i>	Forward TCGCATGATGGACCACAAGA Reverse AGGTACAAGATCCAGCCGC	2.03	(Christen et al., 2019)
	<i>cytochrome c oxidase subunit 5b (cox5b)</i>	Forward TGGATGTGGTTACATGATGGC Reverse AAAGTGGTGCAACTTGAGTAAG	1.95	(Christen et al., 2019)
	<i>cytochrome c oxidase subunit 17 (cox17)</i>	Forward AACCTTGTGTGCTTGT Reverse ATGTGCTTCTATTAAATCCC	2.26	(Christen et al., 2019)
Endocrine regulation	<i>vitellogenin (vtg)</i>	Forward GCAGAATACATGGACGGTGT Reverse GAACAGTCTTCGGAAGCTTG	1.95	(Pankiw and Page, 2000)
	<i>α-glucosidase (hbg-3)</i>	Forward TACCTGGCTTCGTGTCAAC Reverse ATCTTCGGTTTCCCTAGAGAATG	2.06	(Ueno et al., 2015)
	<i>buffy</i>	Forward CATGGCACTTCTCATCCTTTTC Reverse GAGAACGGTTTCAGCATCAATC	2.07	(Ueno et al., 2015)

Christen, V., Krebs, J., Fent, K., 2019. Fungicides chlorothanolin, azoxystrobin and folpet induce transcriptional alterations in genes encoding enzymes involved in oxidative phosphorylation and metabolism in honey bees (*Apis mellifera*) at sublethal concentrations. *Journal of Hazardous Materials* 377, 215–226. <https://doi.org/10.1016/j.jhazmat.2019.05.056>

Evans JD, Aronstein K, Chen YP, Hetru C, Imler JL, Jiang H, Kanost M, Thompson GJ, Zou Z, Hultmark D. 2006. Immune pathways and defense mechanisms in honey bees *Apis mellifera*. *Insect Mol Biol*, 5:645-656. doi: 10.1111/j.1365-2583.2006.00682.x.

Pankiw T, Page RE. 2000. Response thresholds to sucrose predict foraging division of labor in honeybees. *Behavioral Ecology Sociobiol*, 47: 265–267.

Ueno T, Takeuchi H, Kawasaki K, Kubo T. 2015. Changes in the gene expression profiles of the hypopharyngeal gland of worker honeybees in association with worker behavior and hormonal factors, *PLoS One*, 10: e0130206, , <https://doi.org/10.1371/journal.pone.0130206>.

Table S6: RFID homing flight duration of foragers of all treatment groups.

Colony 1	RFID Homing flight duration (return time in seconds)				
	Control	Solvent control	1 ng/bee TMX	2.43 µg/bee Pyr	5.98 µg/bee Pyr
Number of values	11	9	12	8	12
Minimum	397	653	618	363	465
25% Percentile	506	781.5	964	603	687.5
Median	1303	1067	1544	1072	1476
75% Percentile	3195	1654	2124	2335	2023
Maximum	5942	2939	3310	5926	6704
Colony 2					
	Control	Solvent control	1 ng/bee TMX	2.43 µg/bee Pyr	5.98 µg/bee Pyr
Number of values	9	11	8	8	6
Minimum	295	495	259	380	440
25% Percentile	332	671	599.3	476	494.8
Median	604	991	1525	717	1661
75% Percentile	1222	2036	2259	1004	2346
Maximum	2453	2826	3264	1552	2989
Colony 3					
	Control	Solvent control	1 ng/bee TMX	2.43 µg/bee Pyr	5.98 µg/bee Pyr
Number of values	17	10	14	6	1
Minimum	610	900	891	770	4224
25% Percentile	728	1225	1418	829.3	4224
Median	1380	2354	3450	2483	4224
75% Percentile	4517	5401	5646	5196	4224
Maximum	6501	7034	6536	6112	4224

Table S7: Age of foragers of all treatment groups.

		N	mean	standard deviation	standard error	minimum	maximum
age last record	40% Control	55	28.22	4.012	0.541	20	37
	5% Solvent control	52	28.02	4.027	0.558	20	35
	2.43 µg/bee Pyraclostrobin	49	28.08	4.173	0.596	20	37
	5.98 µg/bee Pyraclostrobin	48	27.67	4.759	0.687	20	49
	1 ng/bee Thiamethoxam	40	27.9	3.979	0.629	21	35
	total	244	27.99	4.169	0.267	20	49
days last record after treatment	40% Control	55	5.27	4.232	0.571	0	17
	5% Solvent control	52	5.02	3.908	0.542	0	15
	2.43 µg/bee Pyraclostrobin	49	4.84	3.243	0.463	0	14
	5.98 µg/bee Pyraclostrobin	48	4.98	5.101	0.736	0	29
	1 ng/bee Thiamethoxam	40	4.98	3.952	0.625	1	15
	total	244	5.02	4.102	0.263	0	29

Table S8: Multiple comparisons test of transcript level linked to energy metabolism in foragers performing homing flight.

cox5a									
Number of families	1								
Number of comparisons per family	12								
Alpha	0.05								
Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value					
Control vs. Solvent control	-4.955 No	ns	>0.9999	A-B					
Solvent control vs. 1 ng/bee TMX	7.227 No	ns	>0.9999	B-C					
Solvent control vs. 2.43 µg/bee Pyr	9.875 No	ns	>0.9999	B-D					
Solvent control vs. 5.98 µg/bee Pyr	5.667 No	ns	>0.9999	B-E					
Control vs. Solvent control	-12.58 No	ns	>0.9999	F-G					
Solvent control vs. 1 ng/bee TMX	2.284 No	ns	>0.9999	G-H					
Solvent control vs. 2.43 µg/bee Pyr	7.909 No	ns	>0.9999	G-I					
Solvent control vs. 5.98 µg/bee Pyr	-17.89 No	ns	>0.9999	G-J					
Control vs. Solvent control	-4.063 No	ns	>0.9999	K-L					
Solvent control vs. 1 ng/bee TMX	-4.929 No	ns	>0.9999	L-M					
Solvent control vs. 2.43 µg/bee Pyr	-16.83 No	ns	>0.9999	L-N					
Solvent control vs. 5.98 µg/bee Pyr	13 No	ns	>0.9999	L-O					
Test details	Mean rank 1	Mean rank 2	Mean rank diff.	n1	n2	Z			
Control vs. Solvent control	47.55	52.5	-4.955	11	8	0.2686			
Solvent control vs. 1 ng/bee TMX	52.5	45.27	7.227	8	11	0.3919			
Solvent control vs. 2.43 µg/bee Pyr	52.5	42.63	9.875	8	8	0.4976			
Solvent control vs. 5.98 µg/bee Pyr	52.5	46.83	5.667	8	12	0.3128			
Control vs. Solvent control	43.33	55.91	-12.58	9	11	0.7049			
Solvent control vs. 1 ng/bee TMX	55.91	53.63	2.284	11	8	0.1238			
Solvent control vs. 2.43 µg/bee Pyr	55.91	48	7.909	11	8	0.4288			
Solvent control vs. 5.98 µg/bee Pyr	55.91	73.8	-17.89	11	5	0.8357			
Control vs. Solvent control	100.9	105	-4.063	16	9	0.2456			
Solvent control vs. 1 ng/bee TMX	105	109.9	-4.929	9	14	0.2906			
Solvent control vs. 2.43 µg/bee Pyr	105	121.8	-16.83	9	6	0.8047			
Solvent control vs. 5.98 µg/bee Pyr	105	92	13	9	1	0.3107			
cox5b									
Number of families	1								
Number of comparisons per family	12								
Alpha	0.05								
Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value				
Control vs. Solvent control	-1127 -7752 to 5498	No	ns	>0.9999	A-B				
Solvent control vs. 1 ng/bee TMX	1052 -5573 to 7677	No	ns	>0.9999	B-C				
Solvent control vs. 2.43 µg/bee Pyr	1304 -5825 to 8433	No	ns	>0.9999	B-D				
Solvent control vs. 5.98 µg/bee Pyr	898.4 -5609 to 7406	No	ns	>0.9999	B-E				
Control vs. Solvent control	1712 -4696 to 8120	No	ns	0.9999 F-G					
Solvent control vs. 1 ng/bee TMX	-758.7 -7384 to 5866	No	ns	>0.9999	G-H				
Solvent control vs. 2.43 µg/bee Pyr	-3663 -10288 to 2962	No	ns	0.7527 G-I					
Solvent control vs. 5.98 µg/bee Pyr	-2841 -10532 to 4849	No	ns	0.9818 G-J					
Control vs. Solvent control	-5060 -11001 to 880.7	No	ns	0.1605 K-L					
Solvent control vs. 1 ng/bee TMX	-874.1 -6966 to 5218	No	ns	>0.9999	L-M				
Solvent control vs. 2.43 µg/bee Pyr	4659 -2856 to 12173	No	ns	0.5996 L-N					
Solvent control vs. 5.98 µg/bee Pyr	-4387 -19416 to 10642	No	ns	0.9977 L-O					
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	t	DF	
Control vs. Solvent control	8120	9247	-1127	2275	11	8	0.4954	122	
Solvent control vs. 1 ng/bee TMX	9247	8195	1052	2275	8	11	0.4624	122	
Solvent control vs. 2.43 µg/bee Pyr	9247	7942	1304	2448	8	8	0.5328	122	
Solvent control vs. 5.98 µg/bee Pyr	9247	8348	898.4	2234	8	12	0.4021	122	
Control vs. Solvent control	10011	8299	1712	2200	9	11	0.7781	122	
Solvent control vs. 1 ng/bee TMX	8299	9058	-758.7	2275	11	8	0.3336	122	
Solvent control vs. 2.43 µg/bee Pyr	8299	11962	-3663	2275	11	8	1.61	122	
Solvent control vs. 5.98 µg/bee Pyr	8299	11141	-2841	2640	11	5	1.076	122	
Control vs. Solvent control	17198	22258	-5060	2040	16	9	2.481	122	
Solvent control vs. 1 ng/bee TMX	22258	23132	-874.1	2092	9	14	0.4179	122	
Solvent control vs. 2.43 µg/bee Pyr	22258	17599	4659	2580	9	6	1.806	122	
Solvent control vs. 5.98 µg/bee Pyr	22258	26645	-4387	5160	9	1	0.8501	122	
cox17									
Number of families	1								
Number of comparisons per family	12								
Alpha	0.05								
Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value					
Control vs. Solvent control	-11.11 No	ns	>0.9999	A-B					
Solvent control vs. 1 ng/bee TMX	10.3 No	ns	>0.9999	B-C					
Solvent control vs. 2.43 µg/bee Pyr	16.25 No	ns	>0.9999	B-D					
Solvent control vs. 5.98 µg/bee Pyr	7.667 No	ns	>0.9999	B-E					
Control vs. Solvent control	-12.43 No	ns	>0.9999	F-G					
Solvent control vs. 1 ng/bee TMX	18.92 No	ns	>0.9999	G-H					
Solvent control vs. 2.43 µg/bee Pyr	8.795 No	ns	>0.9999	G-I					
Solvent control vs. 5.98 µg/bee Pyr	-11.05 No	ns	>0.9999	G-J					
Control vs. Solvent control	-18.54 No	ns	>0.9999	K-L					
Solvent control vs. 1 ng/bee TMX	0.09524 No	ns	>0.9999	L-M					
Solvent control vs. 2.43 µg/bee Pyr	-2.667 No	ns	>0.9999	L-N					
Solvent control vs. 5.98 µg/bee Pyr	0.6667 No	ns	>0.9999	L-O					
Test details	Mean rank 1	Mean rank 2	Mean rank diff.	n1	n2	Z			
Control vs. Solvent control	45.64	56.75	-11.11	11	8	0.6026			
Solvent control vs. 1 ng/bee TMX	56.75	46.45	10.3	8	11	0.5582			
Solvent control vs. 2.43 µg/bee Pyr	56.75	40.5	16.25	8	8	0.8188			
Solvent control vs. 5.98 µg/bee Pyr	56.75	49.08	7.667	8	12	0.4232			
Control vs. Solvent control	45.11	57.55	-12.43	9	11	0.697			
Solvent control vs. 1 ng/bee TMX	57.55	38.63	18.92	11	8	1.026			
Solvent control vs. 2.43 µg/bee Pyr	57.55	48.75	8.795	11	8	0.4769			
Solvent control vs. 5.98 µg/bee Pyr	57.55	68.6	-11.05	11	5	0.5164			
Control vs. Solvent control	96.13	114.7	-18.54	16	9	1.121			
Solvent control vs. 1 ng/bee TMX	114.7	114.6	0.09524	9	14	0.005616			
Solvent control vs. 2.43 µg/bee Pyr	114.7	117.3	-2.667	9	6	0.1275			
Solvent control vs. 5.98 µg/bee Pyr	114.7	114	0.6667	9	1	0.01593			

Table S9. Multiple comparisons test of transcript level linked to endocrine systems in foragers performing homing flight test.

buffy						
Number of families	1					
Number of comparisons per family	12					
Alpha	0.05					
Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value		
Control vs. Solvent control	-6.193	No	ns	>0.9999	A-B	
Solvent control vs. 1 ng/bee TMX	-0.8068	No	ns	>0.9999	B-C	
Solvent control vs. 2.43 µg/bee Pyr	22.38	No	ns	>0.9999	B-D	
Solvent control vs. 5.98 µg/bee Pyr	-10.38	No	ns	>0.9999	B-E	
Control vs. Solvent control	-5.96	No	ns	>0.9999	F-G	
Solvent control vs. 1 ng/bee TMX	-11.19	No	ns	>0.9999	G-H	
Solvent control vs. 2.43 µg/bee Pyr	-17.07	No	ns	>0.9999	G-I	
Solvent control vs. 5.98 µg/bee Pyr	-16.42	No	ns	>0.9999	G-J	
Control vs. Solvent control	-9.389	No	ns	>0.9999	K-L	
Solvent control vs. 1 ng/bee TMX	-0.1825	No	ns	>0.9999	L-M	
Solvent control vs. 2.43 µg/bee Pyr	18.56	No	ns	>0.9999	L-N	
Solvent control vs. 5.98 µg/bee Pyr	3.889	No	ns	>0.9999	L-O	
Test details	Mean rank 1	Mean rank 2	Mean rank diff.	n1	n2	Z
Control vs. Solvent control	48.18	54.38	-6.193	11	8	0.3358
Solvent control vs. 1 ng/bee TMX	54.38	55.18	-0.8068	8	11	0.04375
Solvent control vs. 2.43 µg/bee Pyr	54.38	32	22.38	8	8	1.127
Solvent control vs. 5.98 µg/bee Pyr	54.38	64.75	-10.38	8	12	0.5727
Control vs. Solvent control	40.22	46.18	-5.96	9	11	0.334
Solvent control vs. 1 ng/bee TMX	46.18	57.38	-11.19	11	8	0.6069
Solvent control vs. 2.43 µg/bee Pyr	46.18	63.25	-17.07	11	8	0.9254
Solvent control vs. 5.98 µg/bee Pyr	46.18	62.6	-16.42	11	5	0.7669
Control vs. Solvent control	98.5	107.9	-9.389	16	9	0.5677
Solvent control vs. 1 ng/bee TMX	107.9	108.1	-0.1825	9	14	0.01076
Solvent control vs. 2.43 µg/bee Pyr	107.9	89.33	18.56	9	6	0.887
Solvent control vs. 5.98 µg/bee Pyr	107.9	104	3.889	9	1	0.09295
vitellogenin						
Number of families	1					
Number of comparisons per family	12					
Alpha	0.05					
Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value		
Control vs. Solvent control	-7.489	No	ns	>0.9999	A-B	
Solvent control vs. 1 ng/bee TMX	-7.875	No	ns	>0.9999	B-C	
Solvent control vs. 2.43 µg/bee Pyr	10.13	No	ns	>0.9999	B-D	
Solvent control vs. 5.98 µg/bee Pyr	-0.5417	No	ns	>0.9999	B-E	
Control vs. Solvent control	-17.77	No	ns	>0.9999	F-G	
Solvent control vs. 1 ng/bee TMX	9.295	No	ns	>0.9999	G-H	
Solvent control vs. 2.43 µg/bee Pyr	18.55	No	ns	>0.9999	G-I	
Solvent control vs. 5.98 µg/bee Pyr	-2.455	No	ns	>0.9999	G-J	
Control vs. Solvent control	-22.92	No	ns	>0.9999	K-L	
Solvent control vs. 1 ng/bee TMX	21.1	No	ns	>0.9999	L-M	
Solvent control vs. 2.43 µg/bee Pyr	23.83	No	ns	>0.9999	L-N	
Solvent control vs. 5.98 µg/bee Pyr	-3.333	No	ns	>0.9999	L-O	
Test details	Mean rank 1	Mean rank 2	Mean rank diff.	n1	n2	Z
Control vs. Solvent control	54.64	62.13	-7.489	11	8	0.406
Solvent control vs. 1 ng/bee TMX	62.13	70	-7.875	8	11	0.427
Solvent control vs. 2.43 µg/bee Pyr	62.13	52	10.13	8	8	0.5102
Solvent control vs. 5.98 µg/bee Pyr	62.13	62.67	-0.5417	8	12	0.0299
Control vs. Solvent control	48.78	66.55	-17.77	9	11	0.9959
Solvent control vs. 1 ng/bee TMX	66.55	57.25	9.295	11	8	0.504
Solvent control vs. 2.43 µg/bee Pyr	66.55	48	18.55	11	8	1.006
Solvent control vs. 5.98 µg/bee Pyr	66.55	69	-2.455	11	5	0.1147
Control vs. Solvent control	82.75	105.7	-22.92	16	9	1.386
Solvent control vs. 1 ng/bee TMX	105.7	84.57	21.1	9	14	1.244
Solvent control vs. 2.43 µg/bee Pyr	105.7	81.83	23.83	9	6	1.139
Solvent control vs. 5.98 µg/bee Pyr	105.7	109	-3.333	9	1	0.07967
hbg-3						
Number of families	1					
Number of comparisons per family	12					
Alpha	0.05					
Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value		
Control vs. Solvent control	17.48	No	ns	>0.9999	A-B	
Solvent control vs. 1 ng/bee TMX	-30.93	No	ns	>0.9999	B-C	
Solvent control vs. 2.43 µg/bee Pyr	-22	No	ns	>0.9999	B-D	
Solvent control vs. 5.98 µg/bee Pyr	-14.83	No	ns	>0.9999	B-E	
Control vs. Solvent control	16.77	No	ns	>0.9999	F-G	
Solvent control vs. 1 ng/bee TMX	-14.3	No	ns	>0.9999	G-H	
Solvent control vs. 2.43 µg/bee Pyr	12.2	No	ns	>0.9999	G-I	
Solvent control vs. 5.98 µg/bee Pyr	-31.15	No	ns	>0.9999	G-J	
Control vs. Solvent control	-34.56	No	ns	0.4401	K-L	
Solvent control vs. 1 ng/bee TMX	8.913	No	ns	>0.9999	L-M	
Solvent control vs. 2.43 µg/bee Pyr	-3.278	No	ns	>0.9999	L-N	
Solvent control vs. 5.98 µg/bee Pyr	29.56	No	ns	>0.9999	L-O	
Test details	Mean rank 1	Mean rank 2	Mean rank diff.	n1	n2	Z
Control vs. Solvent control	66.73	49.25	17.48	11	8	0.9476
Solvent control vs. 1 ng/bee TMX	49.25	80.18	-30.93	8	11	1.677
Solvent control vs. 2.43 µg/bee Pyr	49.25	71.25	-22	8	8	1.109
Solvent control vs. 5.98 µg/bee Pyr	49.25	64.08	-14.83	8	12	0.8187
Control vs. Solvent control	57.22	40.45	16.77	9	11	0.9399
Solvent control vs. 1 ng/bee TMX	40.45	54.75	-14.3	11	8	0.7751
Solvent control vs. 2.43 µg/bee Pyr	40.45	28.25	12.2	11	8	0.6617
Solvent control vs. 5.98 µg/bee Pyr	40.45	71.6	-31.15	11	5	1.455
Control vs. Solvent control	70	104.6	-34.56	16	9	2.089
Solvent control vs. 1 ng/bee TMX	104.6	95.64	8.913	9	14	0.5256
Solvent control vs. 2.43 µg/bee Pyr	104.6	107.8	-3.278	9	6	0.1567
Solvent control vs. 5.98 µg/bee Pyr	104.6	75	29.56	9	1	0.7064

Table S10: Multiple comparisons test of transcript level linked to energy metabolism in foragers without homing flight.

cox5a									
Number of families		1							
Number of comparisons per family		4							
Alpha		0.05							
Šidák's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value				
Control vs. Solvent control	-7.913	-65.61 to 49.79	No	ns	0.9942	A-B			
Solvent control vs. 1 ng/bee TMX	-39.72	-97.42 to 17.98	No	ns	0.2863	B-C			
Solvent control vs. 2.43 µg/bee Pyr	59.04	1.338 to 116.7	Yes	*	0.043	B-D			
Solvent control vs. 5.98 µg/bee Pyr	40.58	-17.12 to 98.28	No	ns	0.267	B-E			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	t	DF	
Control vs. Solvent control	170.1	178	-7.913	22.24	10	10	0.3558	45	
Solvent control vs. 1 ng/bee TMX	178	217.8	-39.72	22.24	10	10	1.786	45	
Solvent control vs. 2.43 µg/bee Pyr	178	119	59.04	22.24	10	10	2.655	45	
Solvent control vs. 5.98 µg/bee Pyr	178	137.5	40.58	22.24	10	10	1.825	45	
cox5b									
Number of families		1							
Number of comparisons per family		4							
Alpha		0.05							
Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value					
Control vs. Solvent control	-1.9	No	ns	>0.9999	A-B				
Solvent control vs. 1 ng/bee TMX	-8.7	No	ns	0.7281	B-C				
Solvent control vs. 2.43 µg/bee Pyr	11	No	ns	0.3662	B-D				
Solvent control vs. 5.98 µg/bee Pyr	-4.7	No	ns	>0.9999	B-E				
Test details	Mean rank 1	Mean rank 2	Mean rank diff.	n1	n2	Z			
Control vs. Solvent control	23.5	25.4	-1.9	10	10	0.2914			
Solvent control vs. 1 ng/bee TMX	25.4	34.1	-8.7	10	10	1.335			
Solvent control vs. 2.43 µg/bee Pyr	25.4	14.4	11	10	10	1.687			
Solvent control vs. 5.98 µg/bee Pyr	25.4	30.1	-4.7	10	10	0.7209			
cox6c									
Number of families		1							
Number of comparisons per family		4							
Alpha		0.05							
Šidák's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value				
Control vs. Solvent control	-0.0153	-0.08746 to 0.05686	No	ns	0.9703	A-B			
Solvent control vs. 1 ng/bee TMX	-0.03948	-0.1116 to 0.03268	No	ns	0.5083	B-C			
Solvent control vs. 2.43 µg/bee Pyr	0.07411	0.001948 to 0.1463	Yes	*	0.042	B-D			
Solvent control vs. 5.98 µg/bee Pyr	0.07918	0.007014 to 0.1513	Yes	*	0.0263	B-E			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	t	DF	
Control vs. Solvent control	0.1671	0.1824	-0.0153	0.02781	10	10	0.5501	45	
Solvent control vs. 1 ng/bee TMX	0.1824	0.2219	-0.03948	0.02781	10	10	1.42	45	
Solvent control vs. 2.43 µg/bee Pyr	0.1824	0.1083	0.07411	0.02781	10	10	2.665	45	
Solvent control vs. 5.98 µg/bee Pyr	0.1824	0.1032	0.07918	0.02781	10	10	2.847	45	
cox17									
Number of families		1							
Number of comparisons per family		4							
Alpha		0.05							
Šidák's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value				
Control vs. Solvent control	0.4596	-14.18 to 15.10	No	ns	>0.9999	A-B			
Solvent control vs. 1 ng/bee TMX	-22.86	-37.50 to -8.219	Yes	***	0.0008	B-C			
Solvent control vs. 2.43 µg/bee Pyr	1.464	-13.18 to 16.11	No	ns	0.9983	B-D			
Solvent control vs. 5.98 µg/bee Pyr	-4.13	-18.77 to 10.51	No	ns	0.9199	B-E			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	t	DF	
Control vs. Solvent control	12.42	11.96	0.4596	5.644	10	10	0.08143	45	
Solvent control vs. 1 ng/bee TMX	11.96	34.82	-22.86	5.644	10	10	4.051	45	
Solvent control vs. 2.43 µg/bee Pyr	11.96	10.49	1.464	5.644	10	10	0.2593	45	
Solvent control vs. 5.98 µg/bee Pyr	11.96	16.09	-4.13	5.644	10	10	0.7319	45	

Table S11: Multiple comparisons test of transcript level linked to endocrine systems in foragers without homing flight.

vitellogenin									
Number of families	1								
Number of comparisons per family	4								
Alpha	0.05								
Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value					
Control vs. Solvent control	-4.7	No	ns	>0.9999	A-B				
Solvent control vs. 1 ng/bee TMX	-3.4	No	ns	>0.9999	B-C				
Solvent control vs. 2.43 µg/bee Pyr	-7.2	No	ns	>0.9999	B-D				
Solvent control vs. 5.98 µg/bee Pyr	-8.1	No	ns	0.8562	B-E				
Test details	Mean rank 1	Mean rank 2	Mean rank diff.	n1	n2	Z			
Control vs. Solvent control	18	22.7	-4.7	10	10	0.7209			
Solvent control vs. 1 ng/bee TMX	22.7	26.1	-3.4	10	10	0.5215			
Solvent control vs. 2.43 µg/bee Pyr	22.7	29.9	-7.2	10	10	1.104			
Solvent control vs. 5.98 µg/bee Pyr	22.7	30.8	-8.1	10	10	1.242			
hbg-3									
Number of families	1								
Number of comparisons per family	4								
Alpha	0.05								
Šidák's multiple comparisons test	Mean Diff.	95.00% CI of d		Below threshold?	Summary	Adjusted P Value			
Control vs. Solvent control	-207.6	-620.1 to 204.9		No	ns	0.5867	A-B		
Solvent control vs. 1 ng/bee TMX	-19.63	-432.1 to 392.9		No	ns	>0.9999	B-C		
Solvent control vs. 2.43 µg/bee Pyr	152.1	-260.4 to 564.6		No	ns	0.8146	B-D		
Solvent control vs. 5.98 µg/bee Pyr	92.8	-319.7 to 505.3		No	ns	0.9633	B-E		
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	t	DF	
Control vs. Solvent control	566.1	773.7	-207.6	159	10	10	1.306	45	
Solvent control vs. 1 ng/bee TMX	773.7	793.4	-19.63	159	10	10	0.1235	45	
Solvent control vs. 2.43 µg/bee Pyr	773.7	621.6	152.1	159	10	10	0.9566	45	
Solvent control vs. 5.98 µg/bee Pyr	773.7	680.9	92.8	159	10	10	0.5837	45	
buffy									
Number of families	1								
Number of comparisons per family	4								
Alpha	0.05								
Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value					
Control vs. Solvent control	10.8	No	ns	0.3904	A-B				
Solvent control vs. 1 ng/bee TMX	-10	No	ns	0.5002	B-C				
Solvent control vs. 2.43 µg/bee Pyr	-7.3	No	ns	>0.9999	B-D				
Solvent control vs. 5.98 µg/bee Pyr	-12.9	No	ns	0.1914	B-E				
Test details	Mean rank 1	Mean rank 2	Mean rank diff.	n1	n2	Z			
Control vs. Solvent control	28.1	17.3	10.8	10	10	1.657			
Solvent control vs. 1 ng/bee TMX	17.3	27.3	-10	10	10	1.534			
Solvent control vs. 2.43 µg/bee Pyr	17.3	24.6	-7.3	10	10	1.12			
Solvent control vs. 5.98 µg/bee Pyr	17.3	30.2	-12.9	10	10	1.979			

Fig. S1:

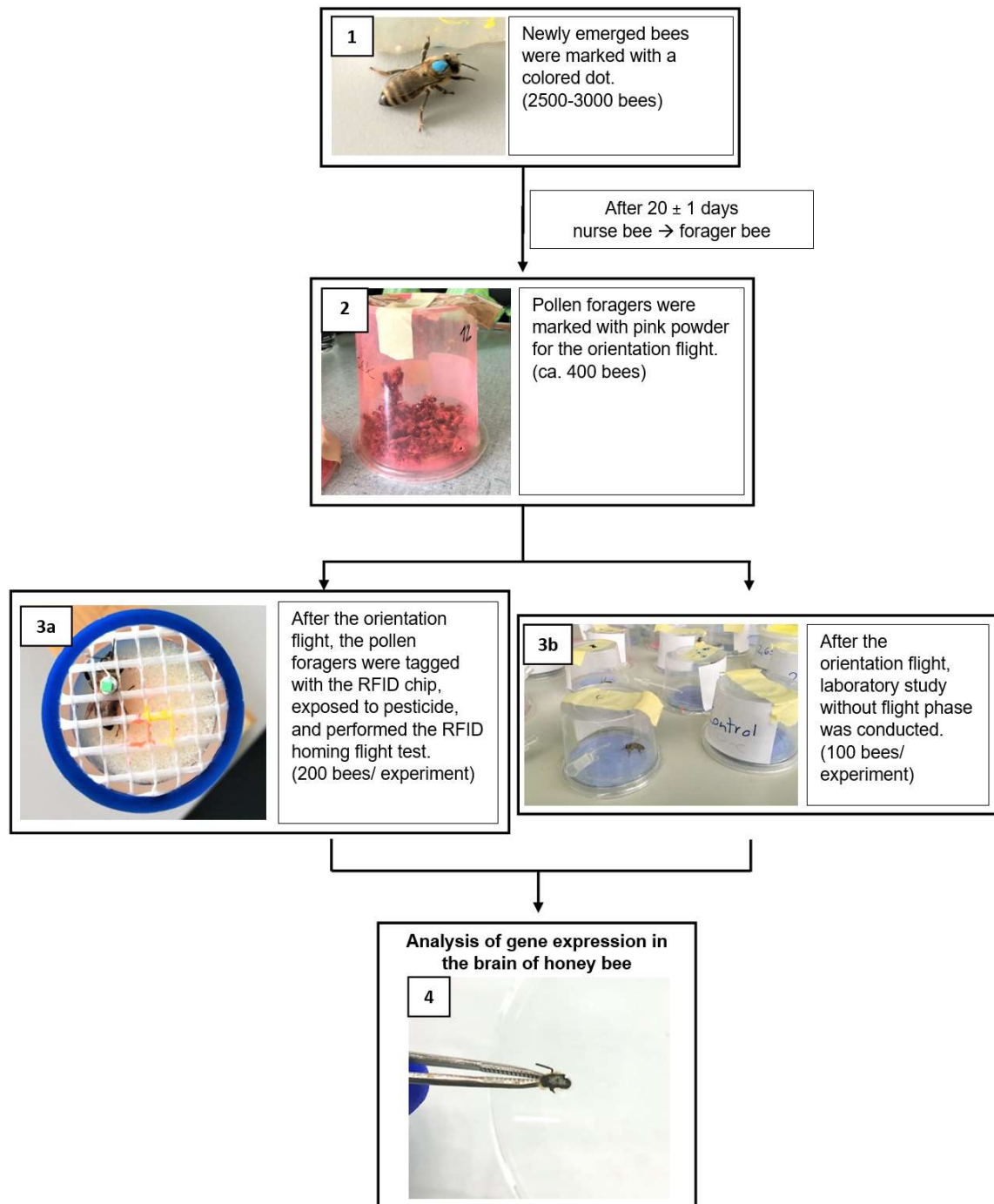


Figure S1: Graphical representation of the RFID experiment. Newly emerged bees were color-coded and then placed back into the colony. At the age of 20-21 days, these marked bees were used for the RFID experiment. They were collected at the flight hole, marked with powder, and released one kilometer from the hive (orientation flight). They were then collected again at the entrance. Now they were either tagged with an RFID chip, exposed to pesticides and released again one kilometer from the hive and during 2 hours returning bees were collected and frozen until gene expression analysis. Or they were only exposed and then frozen until gene expression analysis but without flight (laboratory study).

Fig. S2:

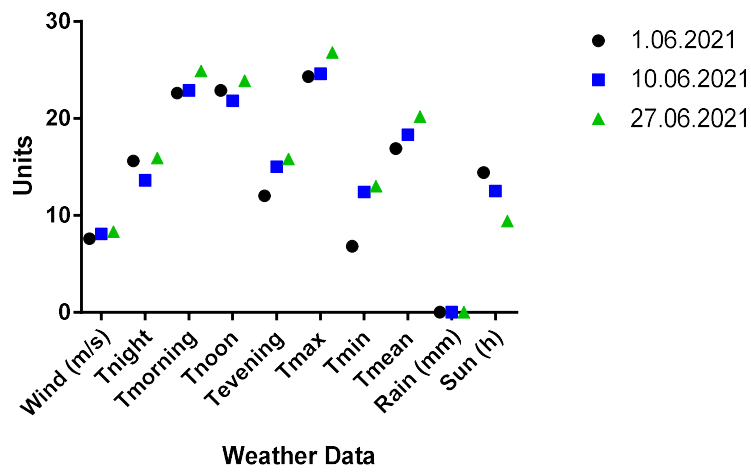


Figure S2: Weather data of the three experimental days. Shown are the maximum wind speed (m/s), the temperature in the night (0.00), in the morning (6 am), at noon (12 am), in the evening (6 pm), the maximum (max), the minimum (min) and the mean temperature at the day in °C, the amount of rain (mm) and the amount of sun (h). Data are from MeteoSchweiz, station Bern Zollikofen.

Fig. S3:



Figure S3: RFID system installed at the entrance of a honeybee colony.

Fig. S4:

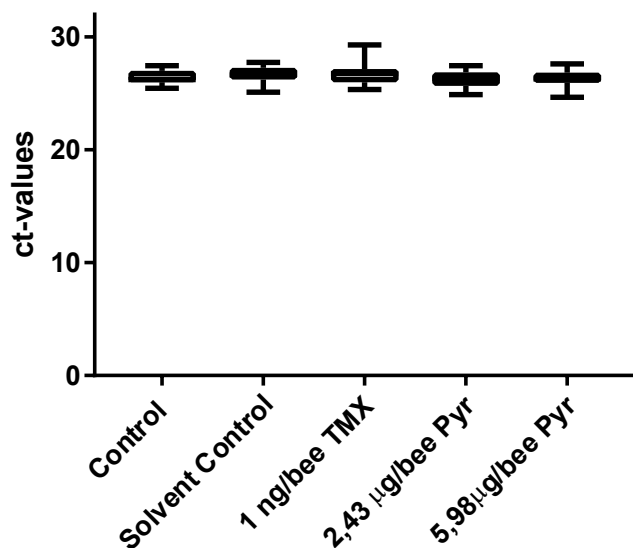


Figure. S4: CT-values of reference transcript *rps5* in the brain of foragers of the sugar control and the four treatment groups from all three colonies. No significant differences were detected.

Fig. S5A-C:

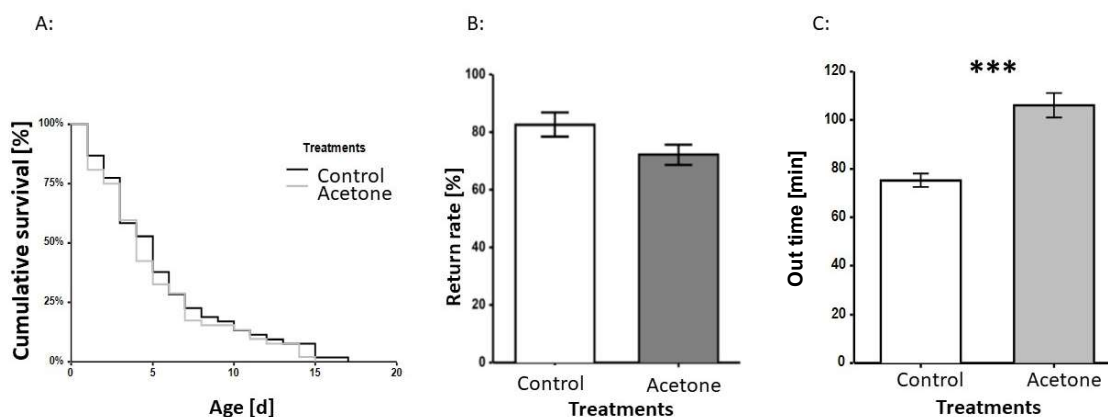


Figure S5: Control vs. acetone in terms of survival, return rate and out hive time of pollen foragers in RFID test. Bees were exposed to sugar solutions (Control) and to 5% acetone control (Acetone), respectively. A) Cumulative survival of foragers of all three colonies. No significant effects between treatment groups. B) In order to determine the return rate, returning bees over 24 hours time period were monitored. No statistically significant differences between treatment groups. C) Out hive time of the foragers of all three colonies. The out hive time was significantly affected by acetone.

Fig. S6:

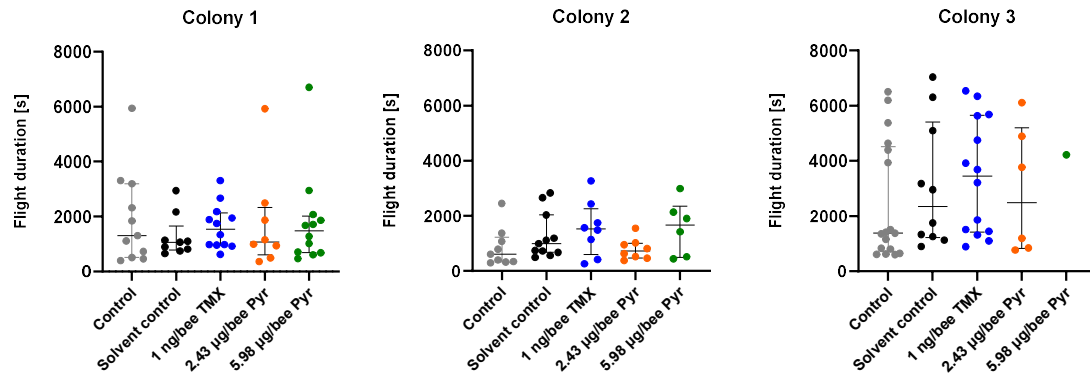


Figure S6: Flight duration in seconds of foragers of the three different colonies after exposure to the four treatment groups (gray: sucrose control, black: solvent control, blue: thiamethoxam, orange and green low respectively high concentration of pyraclostrobin).

Fig. S7:

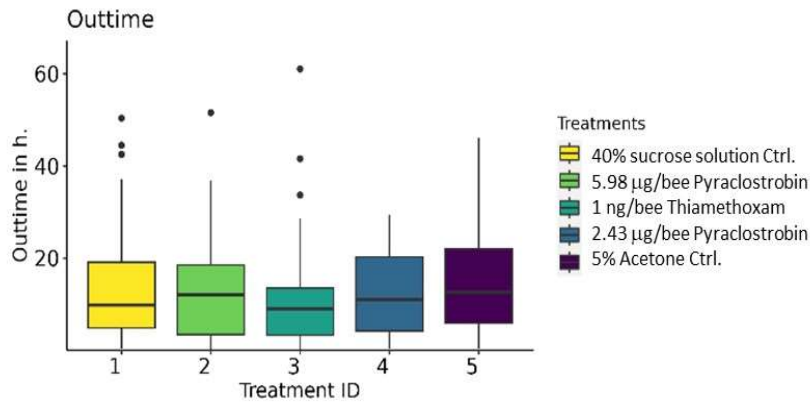


Fig. S7: Out time in hours of foragers of all treatment groups (yellow: sucrose solution control, light green: 5.98 µg/bee pyraclostrobin, dark green: 1 ng/bee thiamethoxam, blue: 2.43 µg/bee pyraclostrobin and dark blue: acetone control) not sunset/sunrise corrected. No significant differences were detected between the different treatment groups.

Fig. S8:

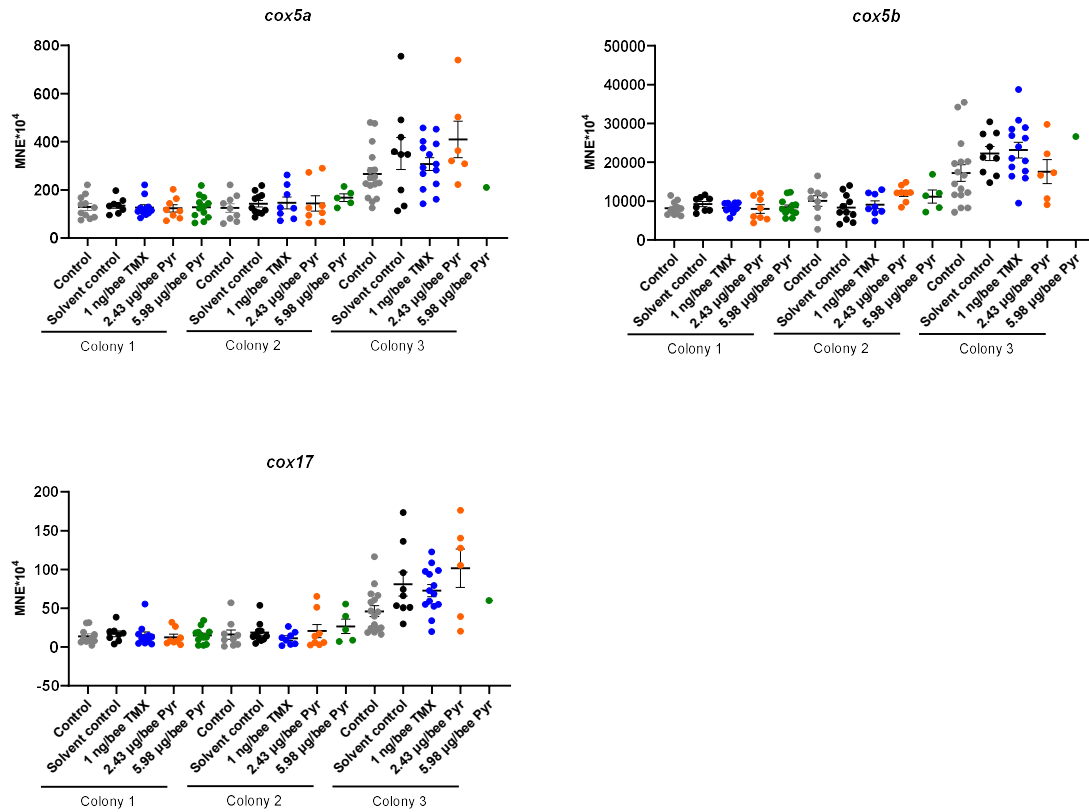


Figure S8: Expression level of *cox5a*, *cox5b* and *cox17* of foragers performing homing flight. Abundance of *cox5a*, *cox5b* and *cox 17* in the brain of foragers of the four treatment groups from all three colonies (grey: sucrose control, black: solvent control, blue: thiamethoxam, orange and green: pyraclostrobin). No significant differences were detected.

Fig. S9:

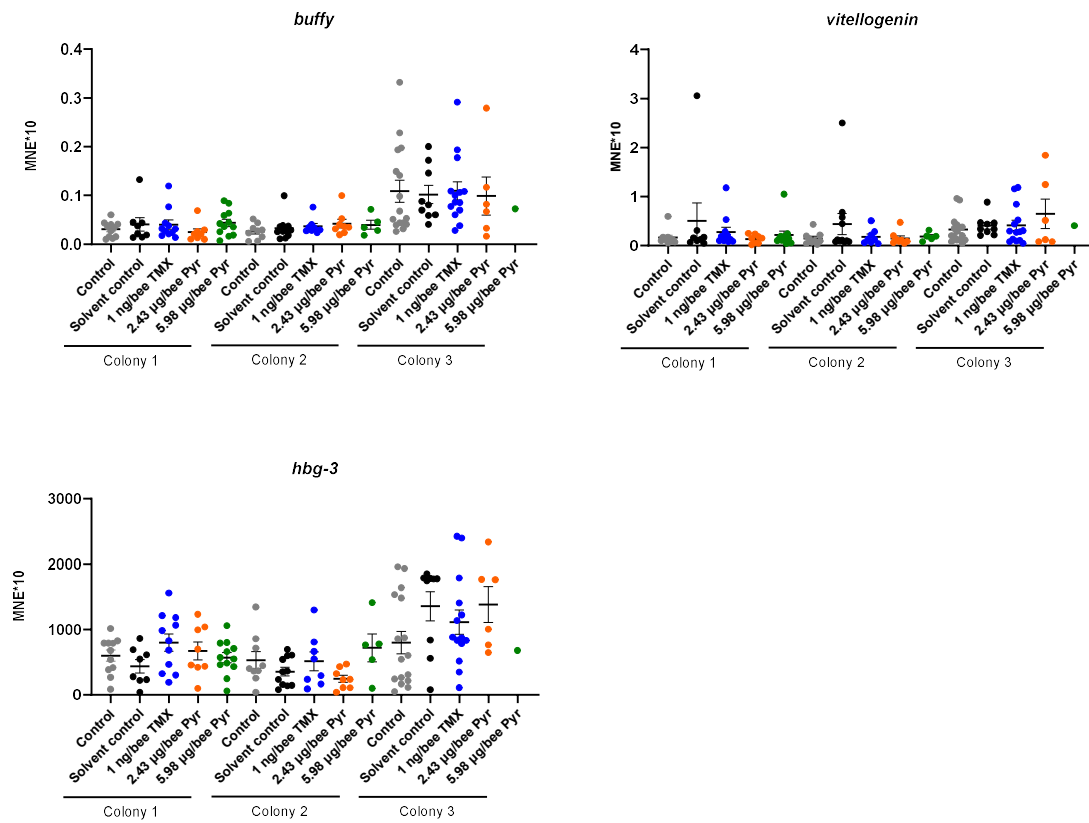


Figure S9: Expression level of transcripts linked to endocrine systems of foragers performing homing flight. Abundance of *buffy*, *hbg-3* and *vitellogenin* in the brain of foragers of the four treatment groups from all three colonies (grey: sucrose control, black: solvent control, blue: thia-methoxam, orange and green: pyraclostrobin). No significant differences were detected.

Fig. S10:

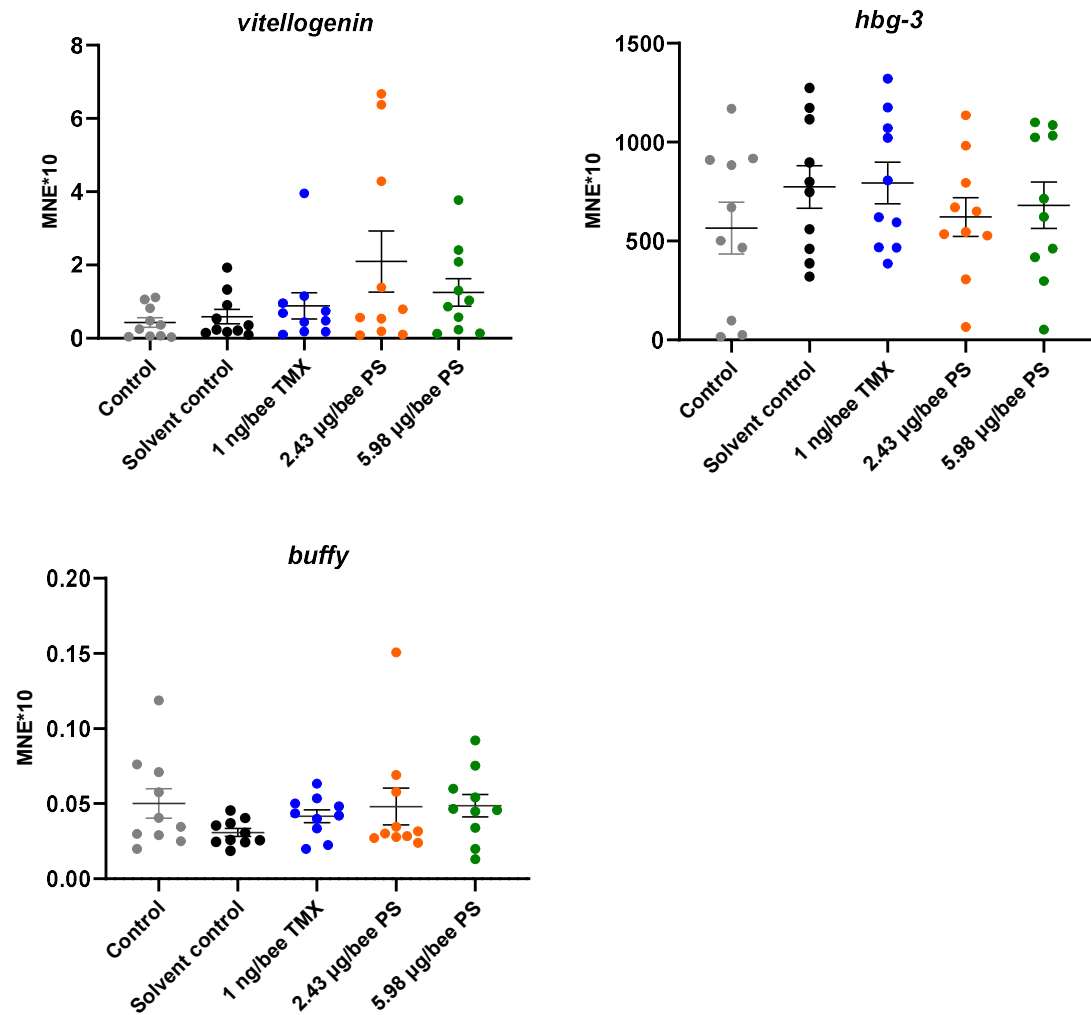


Figure S10: Expression level of *buffy*, *hbg-3* and *vitellogenin* in foragers **without** homing flight. Single dots represent the expression level of individual pollen foragers (n=10 bees per group). Pollen foragers were exposed either to 1ng/bee thiamethoxam or pyraclostrobin at a concentration of 2.43µg/bee or 5.98µg/bee in single feeding without a subsequent flight phase. No significant differences were detected.