

## Supplementary Materials

### Title Interaction Between Thiamethoxam and Deformed Wing Virus type A on Wing Characteristics and Expression of Immune and Apoptosis Genes in *Apis mellifera*

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**Table S1** Primers used for qRT-PCR amplification in this study

Genes	Primer	Sequence 5'-3'	References
Housekeeping			
Ribosomal protein S5 (RPS5)	AmRPS5.F	AATTATTTGGTCGCTGGAATTG	[54]
	AmRPS5.R	TAACGTCCAGCAGAATGTGGTA	
$\beta$ -Actin	Actin.F	TTGTATGCCAACACTGTCCTTT	[55]
	Actin.R	TGGCGCGATGATCTTAATTT	
Immune-related			
Antibacterial peptide abaecin	Abaecin.F	CAGCATTCGCATACGTACCA	[50]
	Abaecin.R	GACCAGGAAACGTTGGAAAC	
Antibacterial peptide defensin	Defensin.F	TGCGCTGCTAACTGTCTCAG	[50]
	Defensin.R	AATGGCACTTAACCGAAACG	
Antibacterial peptide hymenoptaecin	Hymenopt.F	CTCTTCTGTGCCGTTGCATA	[50]
	Hymenopt.R	GCGTCTCCTGTCATTCCATT	
Apoptosis-related			
Apoptotic peptidase activating factor 1-like	Apaf-1.F	ACAGATGATAATTTACAGGTGTGGG	[56]
	Apaf-1.R	TCCGTTCACTCTATCCGTTTGT	
Bcl-2 family proteins-like	Buffy.F	TGCCGATGCCTGAAAAGTCT	[56]
	Buffy.R	TCTGCGATAAGGTTGGCCTG	
Cysteine proteases3-like	Caspase3-like.F	CATGCACAGAAGAAATTCGCCA	[56]
	Caspase3-like.R	GTTCGTCCCGTTTCGTTGTG	
Cysteine proteases8-like	Caspase8-like.F	AAAACAATTGATGCAGTAGGGG	[56]
	Caspase8-like.R	TTCTGGAAATTGAAAATCGGAAGA	
Cysteine proteases9-like	Caspase9-like.F	TGGCCAAAGCTTGTTGAAAATCA	[56]
	Caspase9-like.R	ATGCAAAAGGTCCCCGTGTT	
	DWV.F	CGAAACCAACTTCTGAGGAA	[53]

Deformed wing virus	DWV.R	GTGTTGATCCCTGAGGCTTA	
Black queen cell virus	BQCV-qF7893	AGTGGCGGAGATGTATGC	[46]
	BQCV-qB8150	GGAGGTGAAGTGGCTATATC	
Kashmir bee virus	KBV-qF6639	CCATACCTGCTGATAACC	[46]
	KIABPV-qB6707	CTGAATAATACTGTGCGTATC	
Sacbrood virus	SBV-qF3164	TTGGAACCTACGCATTCTCTG	[46]
	SBV-qB3461	GCTCTAACCTCGCATCAAC	
Acute bee paralysis virus	ABPV-qF6548	TCATACCTGCCGATCAAG	[46]
	KIABPV-qB6707	CTGAATAATACTGTGCGTATC	
Chronic bee paralysis virus	CBPV1-qF1818	CAA CCTGCCTCA ACACAG	[46]
	CBPV1-qB2077	AAT CTGGCAAGG TTGACTGG	
Israeli acute paralysis virus	IAPV.F	CCATGCCTGGCGATTAC	[47]
	IAPV.R	CTGAATAATACTGTGCGTATC	

**Table S2** Statistic of survival in white-eyed pupae honey bees

Treatment		Log-rank test: $X^2$ and <i>p-value</i>			
		control	Thaimethoxam		
			0.001 ng/μl	1.4 ng/μl	14.3 ng/μl
control					
Thiamethoxam	0.001 ng/μl	0.059			
	1.4 ng/μl	<0.0001	0.013		
	14.3 ng/μl	<0.0001	0.028	0.128	

Table S3 Statistic of survival in newly emerged adult honey bees

Treatment		Log-rank test: $X^2$ and <i>p</i> value											
		control			Thiamethoxam								
		control	PBS	DWV	0.001 ng/μl	0.001ng/μl +PBS	0.001 ng/μl+ DWV	1.4 ng/μl	1.4 ng/μl +PBS	1.4 ng/μl DWV	14.3 ng/μl	14.3 ng/μl+ PBS	14.3 ng/μl+ DWV
control	control												
	PBS	0.215											
	DWV	<0.001	0.014										
Thiamethoxam	0.001 ng/μl	0.633	0.110	<0.001									
	0.001ng/μl +PBS	0.351	0.811	0.015	0.189								
	0.001 ng/μl +DWV	<0.001	<0.001	0.012	<0.001	<0.001							
	1.4 ng/μl	0.394	0.865	0.049	0.222	0.976	0.001						
	1.4 ng/μl+ PBS	0.842	0.455	0.016	0.556	0.588	<0.001	0.599					
	1.4 ng/μl+ DWV	<0.001	<0.001	<0.001	<0.001	<0.001	0.310	<0.001	<0.001				
	14.3 ng/μl	<0.001	0.005	0.044	<0.001	<0.001	0.800	0.002	<0.001	0.622			
	14.3 ng/μl +PBS	<0.001	0.016	0.031	<0.001	<0.001	0.649	0.001	<0.001	0.792	0.863		
	14.3 ng/μl +DWV	<0.001	<0.001	<0.001	<0.001	<0.001	0.065	<0.001	<0.001	0.331	0.211	0.316	

**Table S4 Statistic of DWV load in newly emerged adult honey bees**

Games-Howell Post-Hoc Test															
p-value	Characteristic	Treatment	Normal wing						Deformed wing						
			control	PBS	0.001 ng/μl	0.001ng/μl +PBS	1.4 ng/μl	1.4 ng/μl +PBS	DWV	0.001ng/μl	0.001 ng/μl +PBS	0.001 ng/μl +DWV	1.4 ng/μl	1.4 ng/μl +PBS	1.4 ng/μl +DWV
	Normal wing	control													
		PBS	0.962												
		0.001 ng/μl	1.000	1.000											
		0.001 ng/μl+ PBS	1.000	1.000	1.000										
		1.4 ng/μl	0.220	0.774	0.663	0.783									
		1.4 ng/μl+ PBS	0.753	0.936	0.888	0.906	1.000								
	Deformed wing	DWV	0.022	0.022	0.022	0.022	0.022	0.022							
		0.001 ng/μl	0.103	0.664	0.572	0.737	1.000	1.000	0.022						
		0.001 ng/μl +PBS	0.396	0.654	0.572	0.62	0.991	1.000	0.022	0.978					
		0.001ng/μl +DWV	0.014	0.014	0.014	0.014	0.015	0.014	1.000	0.015	0.014				
		1.4 ng/μl	0.031	0.079	0.081	0.167	0.583	0.998	0.022	0.378	1.000	0.015			
1.4 ng/μl +PBS		0.394	0.531	0.473	0.488	0.854	0.995	0.022	0.822	1.000	0.014	1.000			
1.4 ng/μl +DWV		0.010	0.010	0.010	0.010	0.010	0.010	1.000	0.010	0.010	1.000	0.011	0.010		

Table S5 Statistic of *defensin* in newly emerged adult honey bees

Games-Howell Post-Hoc Test												
	C	PBS	DWV	0.001+n	0.001+d	0.001+PBS+n	0.001+PBS+d	0.001+DWV+d	1.4+n	1.4+d	1.4+PBS+n	1.4+PBS+d
<b>PBS</b>	1.000											
<b>DWV</b>	0.003	0.088										
<b>0.001+n</b>	1.000	1.000	0.133									
<b>0.001+d</b>	0.029	0.432	0.026	0.557								
<b>0.001+PBS+n</b>	0.320	0.981	0.002	0.981	0.003							
<b>0.001+PBS+d</b>	0.960	0.980	0.316	0.993	0.985	0.685						
<b>0.001+DWV+d</b>	0.951	0.979	0.258	0.992	0.966	0.639	0.992					
<b>1.4+n</b>	0.066	0.470	0.048	0.596	0.997	0.012	0.596	0.982				
<b>1.4+d</b>	1.000	1.000	0.655	1.000	0.998	0.986	1.000	1.000	0.999			
<b>1.4+PBS+n</b>	0.651	0.849	0.109	0.923	0.943	0.290	0.923	1.000	0.972	1.000		
<b>1.4+PBS+d</b>	1.000	1.000	0.078	1.000	0.485	0.815	1.000	0.998	0.530	1.000	0.949	
<b>1.4+DWV+d</b>	0.179	0.455	0.102	0.587	1.000	0.083	0.587	0.979	1.000	0.998	0.978	0.537

n = normal wing

d = deformed wing

**Table S6** Statistic of *abaecin* in newly emerged adult honey bees

Games-Howell Post-Hoc Test												
	C	PBS	DWV	0.001+n	0.001+d	0.001+PBS+n	0.001+PBS+d	0.001+DWV+d	1.4+n	1.4+d	1.4+PBS+n	1.4+PBS+d
<b>PBS</b>	0.886											
<b>DWV</b>	0.034	0.372										
<b>0.001+n</b>	0.874	1.000	0.103									
<b>0.001+d</b>	0.992	0.771	0.125	0.777								
<b>0.001+PBS+n</b>	0.999	0.940	0.056	0.940	0.932							
<b>0.001+PBS+d</b>	1.000	0.967	0.330	0.981	1.000	1.000						
<b>0.001+DWV+d</b>	0.181	0.990	0.315	0.732	0.374	0.200	0.722					
<b>1.4+n</b>	0.710	1.000	0.711	0.983	0.604	0.777	0.872	1.000				
<b>1.4+d</b>	0.434	0.254	0.059	0.258	0.866	0.367	0.853	0.143	0.203			
<b>1.4+PBS+n</b>	0.667	1.000	0.080	1.000	0.711	0.684	0.968	0.536	0.972	0.261		
<b>1.4+PBS+d</b>	1.000	0.965	0.078	0.981	0.992	1.000	1.000	0.414	0.819	0.439	0.935	
<b>1.4+DWV+d</b>	0.660	1.000	0.454	0.993	0.589	0.736	0.887	1.000	1.000	0.183	0.983	0.815

**n** = normal wing

**d** = deformed wing

**Table S7** Statistic of *hymenoptaecin* in newly emerged adult honey bees

Games-Howell Post-Hoc Test												
	C	PBS	DWV	0.001+n	0.001+d	0.001+PBS+n	0.001+PBS+d	0.001+DWV+d	1.4+n	1.4+d	1.4+PBS+n	1.4+PBS+d
<b>PBS</b>	0.388											
<b>DWV</b>	0.011	0.032										
<b>0.001+n</b>	0.429	0.999	0.161									
<b>0.001+d</b>	0.250	0.700	0.507	0.967								
<b>0.001+PBS+n</b>	0.989	0.554	0.008	0.533	0.289							
<b>0.001+PBS+d</b>	0.349	0.736	0.826	0.945	1.000	0.394						
<b>0.001+DWV+d</b>	0.005	0.116	0.550	0.338	0.837	0.012	0.993					
<b>1.4+n</b>	0.517	0.929	0.738	0.997	1.000	0.582	1.000	0.949				
<b>1.4+d</b>	0.045	0.223	0.316	0.677	1.000	0.045	1.000	0.742	1.000			
<b>1.4+PBS+n</b>	0.004	0.390	0.103	0.916	1.000	0.017	0.999	0.011	1.000	0.815		
<b>1.4+PBS+d</b>	0.325	1.000	0.064	1.000	0.877	0.434	0.866	0.185	0.984	0.411	0.665	
<b>1.4+DWV+d</b>	0.006	0.042	0.999	0.210	0.625	0.005	0.913	0.736	0.826	0.443	0.093	0.086

**n** = normal wing

**d** = deformed wing

**Table S8** Statistic of *caspase3-like* in newly emerged adult honey bees

Games-Howell Post-Hoc Test												
	C	PBS	DWV	0.001+n	0.001+d	0.001+PBS+n	0.001+PBS+d	0.001+DWV+d	1.4+n	1.4+d	1.4+PBS+n	1.4+PBS+d
<b>PBS</b>	0.905											
<b>DWV</b>	0.355	0.193										
<b>0.001+n</b>	0.585	1.000	0.022									
<b>0.001+d</b>	0.246	0.826	0.001	0.603								
<b>0.001+PBS+n</b>	1.000	0.998	0.919	0.984	0.853							
<b>0.001+PBS+d</b>	0.488	0.879	0.208	0.882	0.997	0.819						
<b>0.001+DWV+d</b>	0.774	0.392	1.000	0.291	0.198	0.973	0.219					
<b>1.4+n</b>	0.629	1.000	0.052	1.000	0.773	0.985	0.903	0.287				
<b>1.4+d</b>	0.664	1.000	0.047	1.000	0.673	0.989	0.877	0.302	1.000			
<b>1.4+PBS+n</b>	0.808	1.000	0.025	0.996	0.277	0.998	0.761	0.366	0.997	1.000		
<b>1.4+PBS+d</b>	1.000	0.999	0.547	0.970	0.684	1.000	0.727	0.771	0.974	0.983	0.999	
<b>1.4+DWV+d</b>	0.610	1.000	0.008	1.000	0.330	0.989	0.838	0.312	1.000	1.000	0.999	0.980

**n** = normal wing

**d** = deformed wing



**Table S9** Statistic of *caspase8-like* in newly emerged adult honey bees

Games-Howell Post-Hoc Test												
	C	PBS	DWV	0.001+n	0.001+d	0.001+PBS+n	0.001+PBS+d	0.001+DWV+d	1.4+n	1.4+d	1.4+PBS+n	1.4+PBS+d
<b>PBS</b>	0.993											
<b>DWV</b>	0.071	0.070										
<b>0.001+n</b>	0.392	0.847	0.014									
<b>0.001+d</b>	0.441	0.880	0.019	1.000								
<b>0.001+PBS+n</b>	1.000	0.929	0.033	0.228	0.279							
<b>0.001+PBS+d</b>	0.161	0.466	0.006	0.983	0.983	0.075						
<b>0.001+DWV+d</b>	1.000	1.000	0.046	0.528	0.585	0.994	0.211					
<b>1.4+n</b>	0.768	1.000	0.016	0.745	0.809	0.266	0.277	0.939				
<b>1.4+d</b>	0.140	0.361	0.011	0.875	0.880	0.104	1.000	0.182	0.282			
<b>1.4+PBS+n</b>	1.000	0.999	0.022	0.378	0.446	0.962	0.123	1.000	0.689	0.151		
<b>1.4+PBS+d</b>	0.991	1.000	0.317	1.000	1.000	0.970	0.967	0.999	1.000	0.907	0.998	
<b>1.4+DWV+d</b>	0.514	0.916	0.031	1.000	1.000	0.358	0.985	0.663	0.878	0.896	0.540	1.000

**n** = normal wing

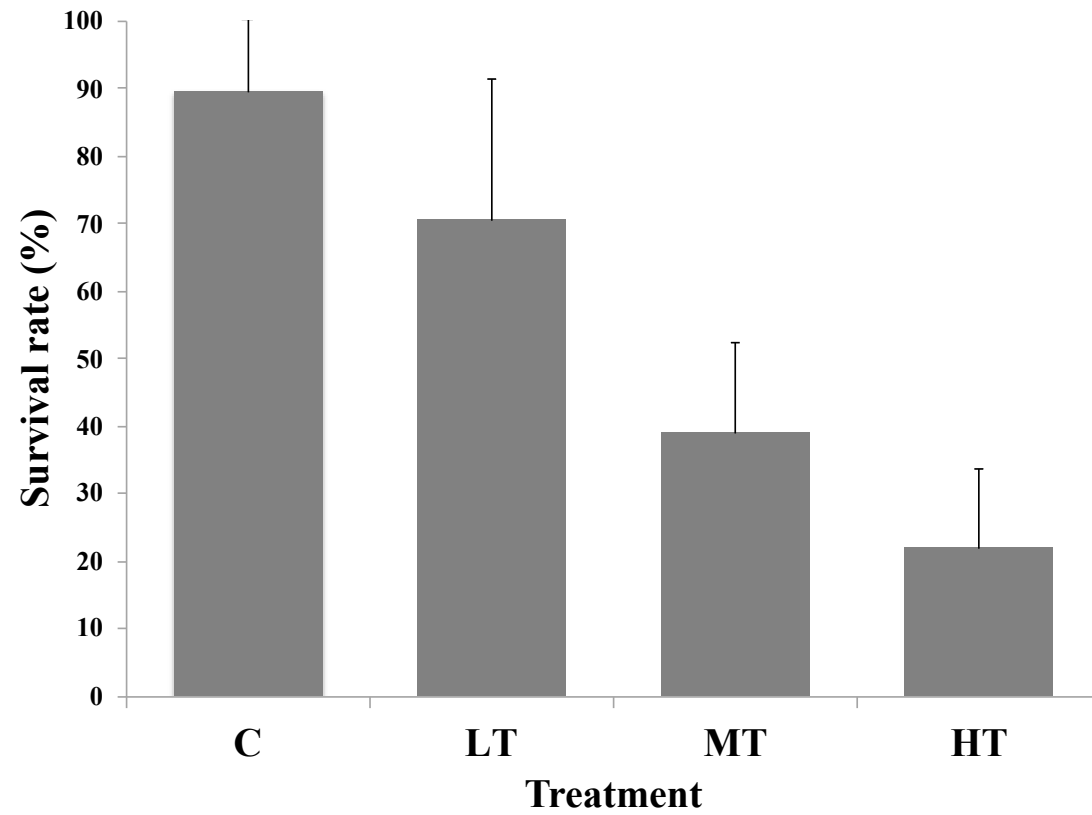
**d** = deformed wing

**Table S10** Statistic of *caspase9-like* in newly emerged adult honey bees

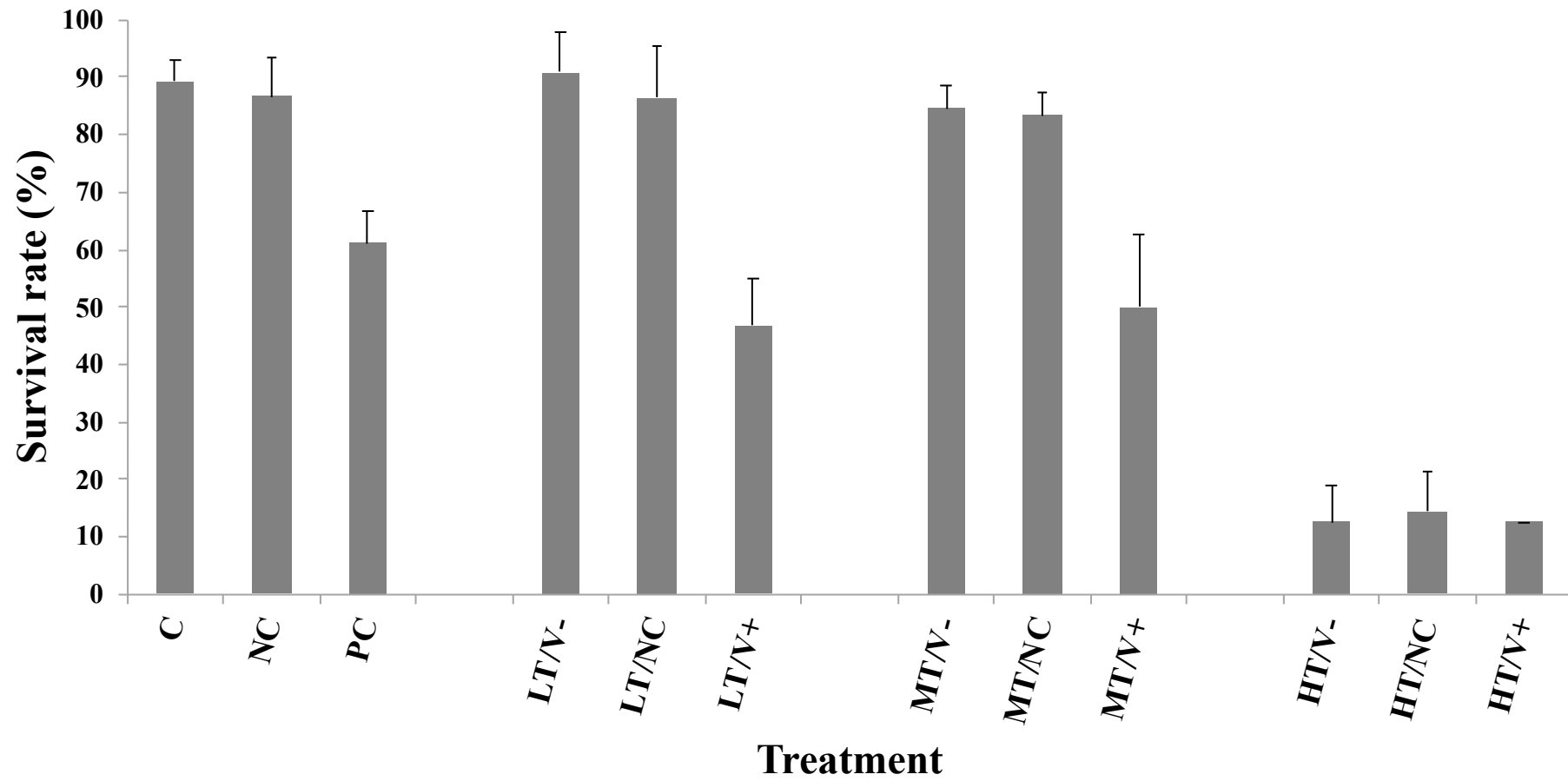
Games-Howell Post-Hoc Test												
	C	PBS	DWV	0.001+n	0.001+d	0.001+PBS+n	0.001+PBS+d	0.001+DWV+d	1.4+n	1.4+d	1.4+PBS+n	1.4+PBS+d
<b>PBS</b>	1.000											
<b>DWV</b>	0.018	0.115										
<b>0.001+n</b>	0.999	1.000	0.036									
<b>0.001+d</b>	0.283	0.794	0.075	0.115								
<b>0.001+PBS+n</b>	1.000	1.000	0.026	1.000	0.187							
<b>0.001+PBS+d</b>	0.987	1.000	0.053	1.000	0.105	1.000						
<b>0.001+DWV+d</b>	0.864	0.999	0.014	0.392	0.052	0.552	0.308					
<b>1.4+n</b>	0.999	1.000	0.014	0.864	0.157	0.956	0.724	0.997				
<b>1.4+d</b>	1.000	1.000	0.098	1.000	0.901	1.000	1.000	0.913	0.993			
<b>1.4+PBS+n</b>	0.999	1.000	0.014	0.865	0.169	0.954	0.732	0.998	1.000	0.993		
<b>1.4+PBS+d</b>	0.600	0.825	0.047	0.496	0.268	0.524	0.470	0.851	0.728	0.612	0.738	
<b>1.4+DWV+d</b>	0.998	1.000	0.026	0.632	0.031	0.872	0.358	0.961	1.000	0.990	1.000	0.683

**n** = normal wing

**d** = deformed wing



**Figure S1.** The percentage of survival rate of white-eyed pupae honey bees at 12 days post-fed.



**Figure S2.** The percentage of survival rates in newly emerged adult honey bees.