

The influence of daily temperature fluctuation on the efficacy of bioinsecticides on Spotted Wing *Drosophila* larvae

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SUPPLEMENTARY MATERIAL

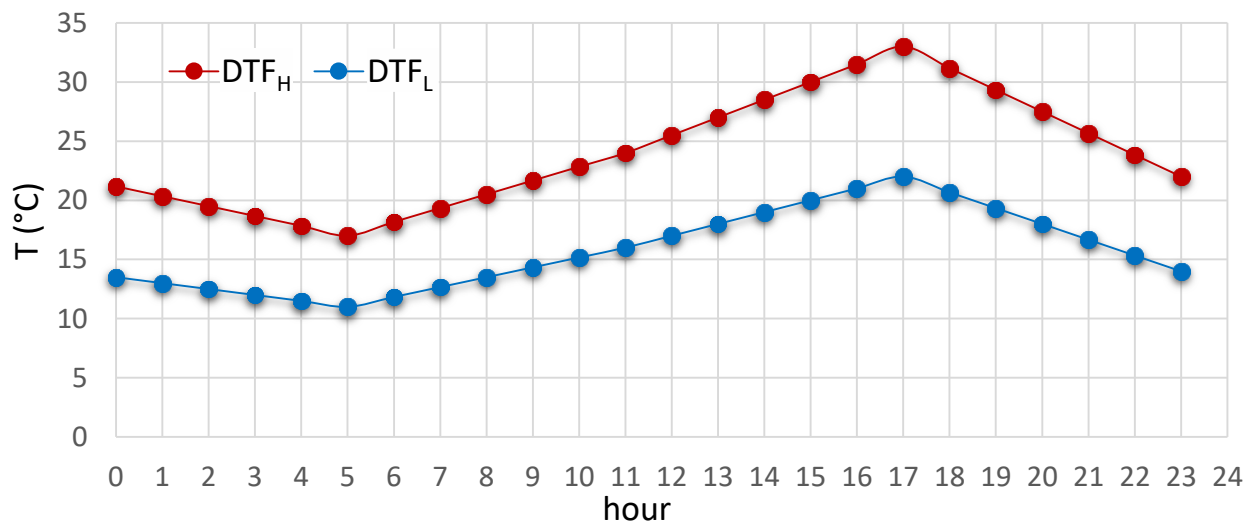


Figure S1. Thermal variation set in the climate chamber over 24 hours in the two temperature ranges, DTF_L 11-22 $^{\circ}\text{C}$ and DTF_H 17-33 $^{\circ}\text{C}$.

Table S1. Pairwise comparisons of SWD mortality (estimated marginal means) after logistic regression (logit model), between different concentrations at the same time and temperature condition. Significant p-values are shown in bold. Cnt = control, C₁-C₅ concentrations of Btk: 640, 1900, 3800, 7600 and 15200 IU/mL, nematodes: 100, 200, 400, 800 and 1600 IJ/mL

		Btk				Sc				Sf				Hb			
		24 h		48 h		24 h		48 h		24 h		48 h		24 h		48 h	
		Z	p	Z	p	Z	p	Z	p	Z	p	Z	P	Z	p	Z	p
CT	Cnt vs C ₁	0.7	1.000	2.4	0.872	4.0	0.031	3.7	0.103	3.8	0.041	6.7	< 0.0001	0.0	1.000	0.0	1.000
	Cnt vs C ₂	5.5	< 0.0001	5.3	0.0002	5.7	< 0.0001	5.7	< 0.0001	6.2	< 0.0001	7.5	< 0.0001	0.9	1.000	0.3	1.000
	Cnt vs C ₃	7.8	< 0.0001	9.8	< 0.0001	8.2	< 0.0001	10.9	< 0.0001	7.1	< 0.0001	8.1	< 0.0001	1.8	0.982	1.0	1.000
	Cnt vs C ₄	10.6	< 0.0001	12.5	< 0.0001	12.6	< 0.0001	14.8	< 0.0001	8.0	< 0.0001	8.8	< 0.0001	3.2	0.191	2.4	0.721
	Cnt vs C ₅	15.1	< 0.0001	20.8	< 0.0001	23.2	< 0.0001	23.3	< 0.0001	8.5	< 0.0001	9.8	< 0.0001	5.9	< 0.0001	4.0	0.020
	C ₁ vs C ₂	4.8	0.002	2.8	0.636	1.6	1.000	2.1	0.983	2.4	0.710	0.8	1.000	0.9	1.000	0.3	1.000
	C ₁ vs C ₃	7.2	< 0.0001	7.3	< 0.0001	4.2	0.021	7.3	< 0.0001	3.4	0.141	1.4	1.000	1.8	0.982	1.0	1.000
	C ₁ vs C ₄	10.0	< 0.0001	10.0	< 0.0001	8.6	< 0.0001	11.1	< 0.0001	4.3	0.009	2.1	0.919	3.2	0.191	2.4	0.721
	C ₁ vs C ₅	14.4	< 0.0001	18.3	< 0.0001	19.2	< 0.0001	19.6	< 0.0001	4.7	0.001	3.1	0.233	5.9	< 0.0001	4.0	0.020
	C ₂ vs C ₃	2.4	0.900	4.5	0.006	2.5	0.826	5.2	0.0003	0.9	1.000	0.5	1.000	0.9	1.000	0.7	1.000
	C ₂ vs C ₄	5.2	0.0003	7.2	< 0.0001	7.0	< 0.0001	9.0	< 0.0001	1.8	0.980	1.3	1.000	2.3	0.802	2.2	0.875
	C ₂ vs C ₅	9.7	< 0.0001	15.5	< 0.0001	17.6	< 0.0001	17.5	< 0.0001	2.3	0.812	2.3	0.786	5.0	0.001	3.8	0.046
	C ₃ vs C ₄	2.8	0.649	2.7	0.714	4.4	0.007	3.8	0.065	0.9	1.000	0.7	1.000	1.4	0.999	1.4	0.999
	C ₃ vs C ₅	7.3	< 0.0001	11.0	< 0.0001	15.0	< 0.0001	12.3	< 0.0001	1.4	0.999	1.8	0.982	4.1	0.015	3.0	0.301
	C ₄ vs C ₅	4.5	0.006	8.3	< 0.0001	10.6	< 0.0001	8.5	< 0.0001	0.5	1.000	1.1	1.000	2.7	0.550	1.6	0.996

		Btk				Sc				Sf			
		24 h		48 h		24 h		48 h		24 h		48 h	
		Z	p	Z	p	Z	p	Z	p	Z	p	Z	p
DTF _L	Cnt vs C ₁	3.5	0.150	4.6	0.003	3.2	0.324	4.8	0.002	1.4	0.999	3.6	0.077
	Cnt vs C ₂	5.4	< 0.0001	6.5	< 0.0001	3.9	0.044	5.4	< 0.0001	4.4	0.006	5.2	0.0003
	Cnt vs C ₃	6.9	< 0.0001	10.2	< 0.0001	5.0	0.001	6.2	< 0.0001	5.0	0.0005	7.0	< 0.0001
	Cnt vs C ₄	8.6	< 0.0001	11.9	< 0.0001	5.8	< 0.0001	7.8	< 0.0001	5.9	< 0.0001	7.7	< 0.0001
	Cnt vs C ₅	13.5	< 0.0001	17.5	< 0.0001	6.4	< 0.0001	10.0	< 0.0001	7.0	< 0.0001	8.4	< 0.0001
	C ₁ vs C ₂	1.9	0.995	1.8	0.998	0.7	1.000	0.6	1.000	3.0	0.322	1.6	0.996
	C ₁ vs C ₃	3.4	0.234	5.5	< 0.0001	1.8	0.998	1.4	1.000	3.6	0.067	3.5	0.103
	C ₁ vs C ₄	5.0	0.001	7.3	< 0.0001	2.6	0.774	3.0	0.463	4.5	0.003	4.2	0.012
	C ₁ vs C ₅	10.0	< 0.0001	12.9	< 0.0001	3.2	0.336	5.2	0.0003	5.6	< 0.0001	4.8	0.001
	C ₂ vs C ₃	1.5	1.000	3.7	0.094	1.0	1.000	0.8	1.000	0.6	1.000	1.9	0.968
	C ₂ vs C ₄	3.1	0.366	5.5	< 0.0001	1.9	0.996	2.4	0.876	1.5	0.997	2.6	0.619
	C ₂ vs C ₅	8.1	< 0.0001	11.1	< 0.0001	2.5	0.870	4.6	0.004	2.6	0.590	3.2	0.188
	C ₃ vs C ₄	1.7	0.999	1.8	0.999	0.8	1.000	1.6	1.000	0.9	1.000	0.7	1.000
	C ₃ vs C ₅	6.7	< 0.0001	7.4	< 0.0001	1.4	1.000	3.8	0.068	2.0	0.948	1.4	1.000
	C ₄ vs C ₅	5.0	0.001	5.6	< 0.0001	0.6	1.000	2.2	0.969	1.1	1.000	0.7	1.000

		Btk				Sc				Hb			
		24 h		48 h		24 h		48 h		24 h		48 h	
		Z	p	Z	p	Z	p	Z	p	Z	p	Z	p
DTF _H	Cnt vs C ₁	4.9	0.001	5.0	0.001	1.8	0.997	7.6	< 0.0001	0.2	1.000	0.3	1.000
	Cnt vs C ₂	6.6	< 0.0001	7.1	< 0.0001	5.4	0.0001	8.3	< 0.0001	0.6	1.000	0.9	1.000
	Cnt vs C ₃	9.5	< 0.0001	11.2	< 0.0001	6.1	< 0.0001	9.0	< 0.0001	1.4	0.999	2.1	0.896
	Cnt vs C ₄	10.5	< 0.0001	13.1	< 0.0001	7.3	< 0.0001	14.0	< 0.0001	3.0	0.335	3.0	0.300
	Cnt vs C ₅	15.1	< 0.0001	17.9	< 0.0001	12.4	< 0.0001	16.4	< 0.0001	3.9	0.030	4.2	0.012
	C ₁ vs C ₂	1.7	0.999	2.1	0.974	3.6	0.140	0.7	1.000	0.3	1.000	0.5	1.000
	C ₁ vs C ₃	4.7	0.003	6.1	< 0.0001	4.3	0.013	1.4	1.000	1.2	1.000	1.8	0.983
	C ₁ vs C ₄	5.6	< 0.0001	8.1	< 0.0001	5.5	< 0.0001	6.4	< 0.0001	2.7	0.489	2.7	0.545
	C ₁ vs C ₅	10.2	< 0.0001	12.8	< 0.0001	10.6	< 0.0001	8.8	< 0.0001	3.7	0.059	3.8	0.039
	C ₂ vs C ₃	3.0	0.515	4.0	0.035	0.7	1.000	0.7	1.000	0.9	1.000	1.3	1.000
	C ₂ vs C ₄	3.9	0.051	5.9	< 0.0001	1.9	0.994	5.7	< 0.0001	2.4	0.747	2.1	0.886
	C ₂ vs C ₅	8.5	< 0.0001	10.7	< 0.0001	7.0	< 0.0001	8.1	< 0.0001	3.3	0.153	3.3	0.167
	C ₃ vs C ₄	0.9	1.000	1.9	0.994	1.2	1.000	5.0	0.001	1.5	0.998	0.9	1.000
	C ₃ vs C ₅	5.6	< 0.0001	6.7	< 0.0001	6.3	< 0.0001	7.4	< 0.0001	2.4	0.712	2.0	0.931
	C ₄ vs C ₅	4.6	0.004	4.8	0.002	5.1	0.001	2.3	0.921	0.9	1.000	1.1	1.000

Table S2. Pairwise comparisons of SWD mortality (estimated marginal means) after logistic regression (logit model), between different times at the same concentration and temperature condition. Significant p-values are shown in bold. Cnt = control, C₁-C₅ concentrations of Btk: 640, 1900, 3800, 7600 and 15200 IU/mL, nematodes: 100, 200, 400, 800 and 1600 IJ/mL

		Btk		Sc		Sf		Hb	
		24 h vs 48 h		24 h vs 48 h		24 h vs 48 h		24 h vs 48 h	
		Z	p	Z	p	Z	p	Z	p
CT	Cnt	2.6	0.769	2.9	0.564	4.5	0.004	3.7	0.059
	C ₁	4.4	0.008	2.5	0.840	7.4	< 0.0001	3.7	0.059
	C ₂	2.4	0.887	3.0	0.508	5.7	< 0.0001	3.0	0.305
	C ₃	4.5	0.005	5.6	< 0.0001	5.4	0.0001	2.9	0.393
	C ₄	4.4	0.007	5.0	0.001	5.2	0.0002	2.9	0.394
	C ₅	8.3	< 0.0001	2.9	0.516	5.8	< 0.0001	1.8	0.981
DTF _L	Cnt	0.3	1.000	0.4	1.000	0.4	1.000		
	C ₁	1.4	1.000	2.0	0.986	2.6	0.588		
	C ₂	1.4	1.000	1.9	0.996	1.2	1.000		
	C ₃	3.6	0.122	1.6	1.000	2.5	0.701		
	C ₄	3.7	0.103	2.4	0.876	2.3	0.834		
	C ₅	4.3	0.012	4.0	0.033	1.8	0.974		
DTF _H	Cnt	1.5	1.000	1.8	0.997			1.5	0.998
	C ₁	1.6	1.000	7.6	< 0.0001			1.7	0.993
	C ₂	2.0	0.989	4.8	0.002			1.8	0.976
	C ₃	3.1	0.421	4.7	0.002			2.2	0.855
	C ₄	4.1	0.031	8.6	< 0.0001			1.6	0.996
	C ₅	4.2	0.017	5.8	< 0.0001			1.8	0.981

Table S3. Pairwise comparisons of SWD mortality (estimated marginal means) after logistic regression (logit model), between different temperature condition at the same time and concentration. Significant p-values are shown in bold. Cnt = control, C₁-C₅ concentrations of Btk: 640, 1900, 3800, 7600 and 15200 IU/mL, nematodes: 100, 200, 400, 800 and 1600 IJ/mL

			Btk		Sc		Sf		Hb	
			Z	p	Z	p	Z	p	Z	p
CT vs DTF _L	24 h	Cnt	0.7	1.000	0.4	1.000	1.5	0.997		
		C ₁	3.5	0.150	0.4	1.000	0.9	1.000		
		C ₂	0.3	1.000	1.3	1.000	0.3	1.000		
		C ₃	0.6	1.000	2.8	0.643	0.6	1.000		
		C ₄	1.4	1.000	6.4	< 0.0001	0.6	1.000		
		C ₅	0.9	1.000	16.4	< 0.0001	0.0	1.000		
	48 h	Cnt	1.6	1.000	2.0	0.985	2.5	0.667		
		C ₁	0.6	1.000	0.9	1.000	5.6	< 0.0001		
		C ₂	0.4	1.000	2.4	0.902	4.8	0.001		
		C ₃	1.2	1.000	6.8	< 0.0001	3.5	0.092		
		C ₄	2.2	0.968	9.0	< 0.0001	3.5	0.086		
		C ₅	4.9	0.001	15.3	< 0.0001	3.9	0.025		
CT vs DTF _H	24 h	Cnt	1.7	0.999	1.8	0.998			2.4	0.744
		C ₁	6.0	< 0.0001	0.4	1.000			2.6	0.584
		C ₂	2.9	0.567	1.5	1.000			2.1	0.924
		C ₃	3.4	0.186	0.3	1.000			2.1	0.924
		C ₄	1.6	1.000	3.5	0.146			2.1	0.891
		C ₅	1.7	0.999	9.0	< 0.0001			0.4	1.000
	48 h	Cnt	0.6	1.000	0.7	1.000			0.3	1.000
		C ₁	3.1	0.365	4.7	0.003			0.6	1.000
		C ₂	2.5	0.857	3.3	0.252			0.9	1.000
		C ₃	2.0	0.991	1.2	1.000			1.4	0.999
		C ₄	1.2	1.000	0.02	1.000			0.8	1.000
		C ₅	2.3	0.923	6.2	< 0.0001			0.4	1.000
DTF _L vs DTF _H	24 h	Cnt	1.1	1.000	1.4	1.000				
		C ₁	2.4	0.884	0.0	1.000				
		C ₂	2.2	0.951	2.8	0.619				
		C ₃	3.7	0.083	2.5	0.839				
		C ₄	3.0	0.478	2.9	0.582				
		C ₅	2.6	0.764	7.4	< 0.0001				
	48 h	Cnt	2.2	0.960	2.8	0.649				
		C ₁	2.6	0.792	5.6	< 0.0001				
		C ₂	2.9	0.560	5.7	< 0.0001				
		C ₃	3.2	0.324	5.6	< 0.0001				
		C ₄	3.4	0.219	9.0	< 0.0001				
		C ₅	2.5	0.822	9.2	< 0.0001				

Table S4. Lethal concentrations (LC₅₀ and LC₉₀) of Btk on SWD at 48 h for all temperature conditions, calculated by Probit analysis with 95% confidence intervals (CI_{95%}). Significant p-values are shown in bold.

	LC ₅₀	CI _{95%} - LC ₅₀	LC ₉₀	CI _{95%} - LC ₉₀	χ^2	p
CT	4876	4363-5445	10713	9693-12038	185	< 0.0001
DTF_L	6768	6039-7589	15520	13980-17543	176	< 0.0001
DTF_H	3915	3286-4558	11800	10548-13463	156	< 0.0001