

SUPPLEMENTARY INFORMATION

Improved efficiency of an herbicide combining bentazone and terbuthylazine – can weeds be controlled with better environmental safety?

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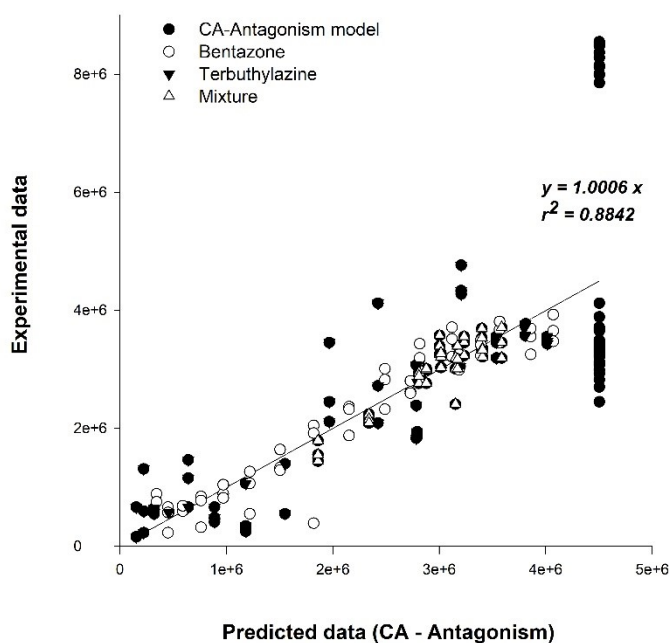


Figure S1. Regression between the experimental responses obtained in the mixture assay with *Raphidocelis subcapitata* and corresponding data predicted by the reference CA–Antagonism model following the integration of single-herbicide concentration-response experimental data.

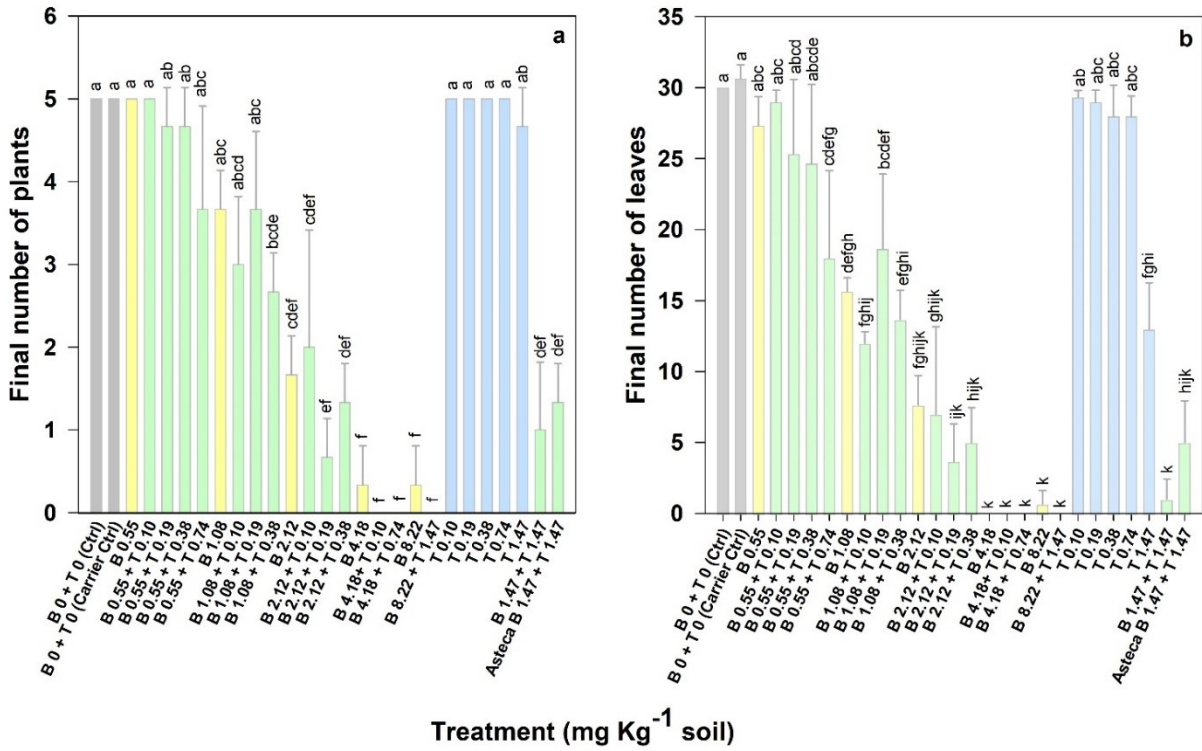


Figure S2. Final number of plants (a) and leaves (b) of *Brassica napus* following a 14-days exposure period to bentazone (B) and terbuthylazine (T), singly and in mixture, or to Asteca® Mays diluted to the same concentration tested in one of the mixture treatments. The bars stand for the average of three replicates and the error bars for the respective standard error. Significant differences are presented for each treatment (Tukey’s test, $p < 0.05$) using low-case letters.

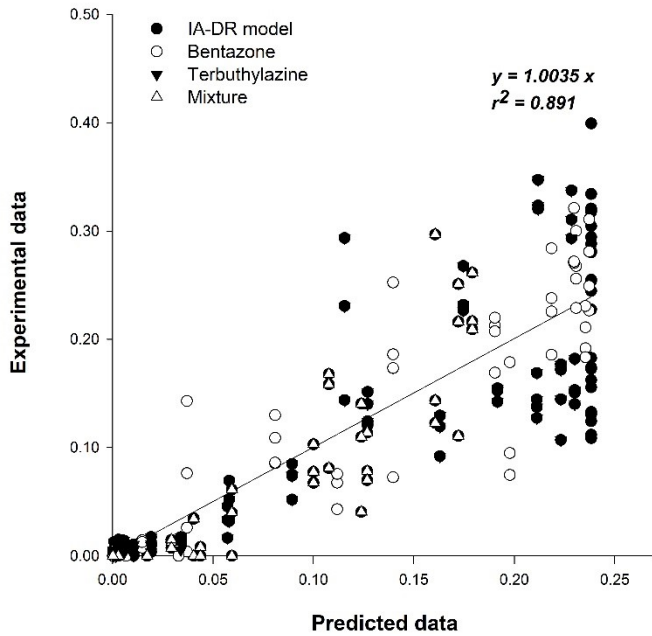


Figure S3. Regression between the experimental responses obtained in the mixture assay with *Brassica napus* and corresponding data predicted by the reference IA – Dose ratio model following the integration of single-herbicide concentration-response experimental data.

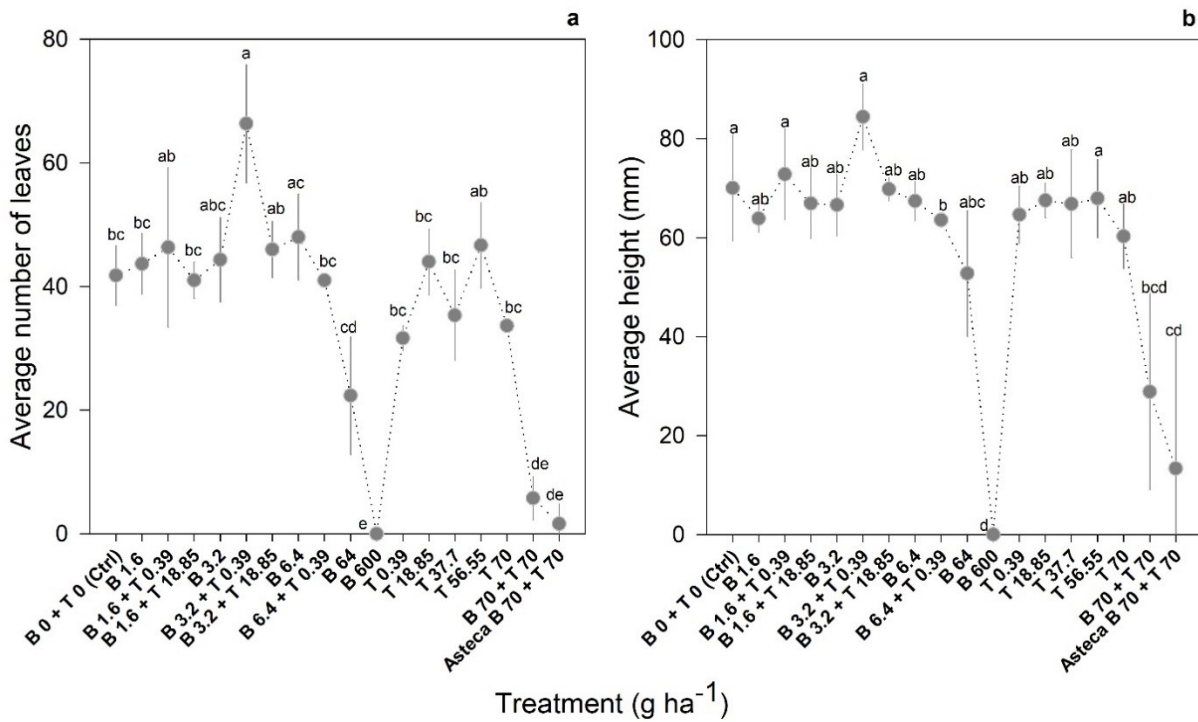


Figure S4. Final total number of leaves (a) and height (b) of *Portulaca oleracea* following a 28-days exposure period to bentazone (B) and terbutylazine (T), singly and in mixture, or to Asteca® Mays diluted to the same concentration tested in one of the other treatments. The marks represent the average of three replicates and the error bars stand for the respective standard error. Significant differences among treatments (Tukey's test, $p < 0.05$) are presented using low-case letters.

Table S1. Concentrations of bentazone (B) and terbuthylazine (T) tested in single/mixture exposures with aquatic (*Lemna gibba*, *Raphidocelis subcapitata*) and terrestrial (*Triticum aestivum*, *Brassica napus*) non-target organisms. Concentrations are given in $\mu\text{g L}^{-1}$ (aquatic tests) or mg Kg^{-1} d.w. (soil tests), as well as in dimensionless Toxic Units (TU) scaling in the specific case of mixtures testing. The treatment in bold corresponds to the commercial formulation Asteca® Mays that was diluted to the same concentrations of B and T as tested in another mixture treatment.

Non-target organisms' exposures																
Aquatic								Terrestrial								
<i>Lemna gibba</i>		<i>Raphidocelis subcapitata</i>						<i>Triticum aestivum</i>		<i>Brassica napus</i>						
Single	Single		Mixture				Σ TU	Single		Single		Mixture				
B	B	T*	B		T			B	T	B	T	B		T		
$\mu\text{g L}^{-1}$	$\mu\text{g L}^{-1}$		$\mu\text{g L}^{-1}$	TU	$\mu\text{g L}^{-1}$	TU	mg Kg^{-1} d.w.		mg Kg^{-1} d.w.		mg Kg^{-1} d.w.	TU	mg Kg^{-1} d.w.	TU	Σ TU	
0	0	0	0	0.000	0.000	0.000	0.000	0.00	0.00	0.00	0.00	0.000	0.000	0.000	0.000	0.000
900	2000	15	0	0.000	6.631	0.239	0.239	0.40	0.83	0.23	0.09	0.000	0.000	0.098	0.136	0.136
1440	2400	20	0	0.000	8.727	0.315	0.315	0.55	1.16	0.35	0.13	0.000	0.000	0.192	0.268	0.268
2304	2880	25	0	0.000	11.486	0.414	0.414	0.77	1.63	0.52	0.20	0.000	0.000	0.378	0.528	0.528
3686	3456	33	0	0.000	15.116	0.545	0.545	1.08	2.28	0.78	0.29	0.000	0.000	0.744	1.040	1.040
5898	4147	43	0	0.000	19.894	0.717	0.717	1.52	3.19	1.17	0.44	0.000	0.000	1.465	2.046	2.046
9437	4977	56	1009	0.257	0.000	0.000	0.257	2.13	4.46	1.76	0.66	0.548	0.271	0.000	0.000	0.271
15099	5972	72	1328	0.338	0.000	0.000	0.338	2.98	6.25	2.63	0.99	1.078	0.534	0.000	0.000	0.534
	7166	94	1747	0.445	0.000	0.000	0.445	4.17	8.75	3.95	1.48	2.122	1.050	0.000	0.000	1.050
	8600	122	2300	0.585	0.000	0.000	0.585	5.83	12.24	5.93	2.22	4.176	2.066	0.000	0.000	2.066
	10320	159	3026	0.770	0.000	0.000	0.770	8.17	17.14	8.89	3.34	8.219	4.067	0.000	0.000	4.067
	12383	207	1009	0.257	6.630	0.239	0.496	11.43	24.00	13.33	5.00	0.548	0.271	0.098	0.136	0.408
	14860	269	1328	0.338	6.630	0.239	0.577			20.00	7.51	1.078	0.534	0.098	0.136	0.670
	17832		1747	0.445	6.630	0.239	0.684			30.00	11.26	2.122	1.050	0.098	0.136	1.186
			2300	0.585	6.630	0.239	0.824				16.89	4.176	2.066	0.098	0.136	2.203
			1009	0.257	8.727	0.315	0.571				25.33	0.548	0.271	0.192	0.268	0.540
			1328	0.338	8.727	0.315	0.652				38.00	1.078	0.534	0.192	0.268	0.802
			1747	0.445	8.727	0.315	0.759					2.122	1.050	0.192	0.268	1.318
			1009	0.257	11.486	0.414	0.671					0.548	0.271	0.378	0.528	0.799
			1328	0.338	11.486	0.414	0.752					1.078	0.534	0.378	0.528	1.062
			1747	0.445	11.486	0.414	0.858					2.122	1.050	0.378	0.528	1.578
			1009	0.257	15.116	0.545	0.801					0.548	0.271	0.744	1.040	1.311
			2300	0.585	15.116	0.545	1.130					4.176	2.066	0.744	1.040	3.106
			3026	0.770	19.894	0.717	1.487					8.219	4.067	1.465	2.046	6.113
			30.000	0.008	0.000	0.000	0.008									
			0.000	0.000	30.000	1.081	1.081									
			30.000	0.008	30.000	1.081	1.089					1.465	0.725	1.465	2.046	2.771
			30.000	0.008	30.000	1.081	1.089					1.465	0.725	1.465	2.046	2.771

*Test made in a previous study of Queirós et al. (2018b) that was considered to plan the mixture assay performed herein

Table S2. Application doses (g ha^{-1}) of bentazone (B) and terbuthylazine (T) tested in the mixture assay with the target weed *Portulaca oleracea*. Predicted environmental concentrations (PECs) corresponding to these application doses are given in $\mu\text{g L}^{-1}$ (surface water) or mg Kg^{-1} d.w. (soil). One of the tested application doses of T results in $\text{PEC}_{\text{water}}$ equal to the EC_{20} value calculated for *Raphidocelis subcapitata* in the single toxicity test, as indicated within brackets. The treatment in bold corresponds to the commercial formulation Asteca® Mays that was diluted to the same concentrations of B and T as tested in another mixture treatment.

Target weed exposure (<i>Portulaca oleracea</i>)					
Mixture					
B			T		
Application dose	$\text{PEC}_{\text{water}}$	PEC_{soil}	Application dose	$\text{PEC}_{\text{water}}$	PEC_{soil}
0.00	0.00	0.00	0.00	0.00	0.00
1.60	0.53	0.01	0.00	0.00	0.00
3.20	1.05	0.02	0.00	0.00	0.00
6.40	2.11	0.05	0.00	0.00	0.00
64.00	21.09	0.48	0.00	0.00	0.00
600.00**	197.76	4.50	0.00	0.00	0.00
0.00	0.00	0.00	0.39	0.11	0.00
0.00	0.00	0.00	18.85	5.40	0.14
0.00	0.00	0.00	37.70	(EC_{20}) 10.82	0.28
0.00	0.00	0.00	56.55	16.21	0.42
0.00	0.00	0.00	70.00	20.07	0.53
1.60	0.53	0.01	0.39	0.11	0.00
1.60	0.53	0.01	18.85	5.40	0.14
3.20	1.05	0.02	0.39	0.11	0.00
3.20	1.05	0.02	18.85	5.40	0.14
6.40	2.11	0.05	0.39	0.11	0.00
70.00	23.07	0.53	70.00	20.07	0.53
70.00	23.07	0.53	70.00	20.07	0.53

**Recommended application dose of T in the commercial formulation

Table S3. Values used to complete the parametrization required at step 1/2 of the FOCUS Surface water tool (Linders *et al.*, 2001) and PERSAM Soil tool (EFSA, 2015; Gardi *et al.*, 2011; Hiederer, 2012) for estimating PEC values in surface water and in soil, respectively, according with the pesticide

application doses. Substance specific chemical data was retrieved from (EFSA, 2011) for terbuthylazine and (EFSA, 2015b) for bentazone.

	FOCUS surface water, step 1	PERSAM soil, step 1	
Bentazone	Molecular weight (g mol ⁻¹)	—	240.3
	Water solubility (g L ⁻¹)	0.570	—
	K _{OC} /K _{foc} (L kg ⁻¹)	30.2	30.2
	DT ₅₀ in soil (days)	7.5	7.5
	DT ₅₀ in water/sediment system (days)	242	—
	DT ₅₀ in water (days)	242	—
	DT ₅₀ in sediment (days)	242	—
	K _{om} (mL g ⁻¹)	—	17.52
	E (KJ mol ⁻¹)	—	65.4
Application pattern (crop, region and season of application, number of applications <i>per</i> season)	Maize, Southern Europe, spring, 1 app/season	South, annual crop	
t _{avg} (days; averaging time)	—	0	
t _{PostApp} (days; time post-application)	—	0	
Z _{eco} (total soil; cm; Ecotoxicological averaging depth)	—	5	
Application doses (g ha ⁻¹)	1, 2, 20, 200, 400, 600, 800, 1000, 1200	20, 200, 400, 600, 800, 1000	
Terbuthylazine	Molecular weight (g mol ⁻¹)	—	229.7
	Water solubility (g L ⁻¹)	0.0085	—
	K _{OC} /K _{foc} (L kg ⁻¹)	151	151
	DT ₅₀ in soil (days)/DgT50 (days)	19.4	19.4
	DT ₅₀ in water/sediment system (days)	69.9	—
	DT ₅₀ in water (days)	1000	—
	DT ₅₀ in sediment (days)	69.9	—
	K _{om} (mL g ⁻¹)	—	87.59
	E (KJ mol ⁻¹)	—	65.4
Application pattern (crop, region and season of application, number of applications <i>per</i> season)	Maize, Southern Europe, spring, 1 app/season	South, annual crop	
t _{avg} (days; averaging time)	—	0	

t_{PostApp} (days; time post-application)	—	0
Z_{eco} (total soil; cm; Ecotoxicological averaging depth)	—	5
Application doses (g ha^{-1})	1, 2, 20, 200, 400, 600, 800, 1000, 1200	20, 200, 400, 600, 800, 1000

Table S4. ECx values calculated in the mixture assays with the non-target aquatic and terrestrial representatives (*Raphidocelis subcapitata* and *Brassica napus*, respectively) and respective conversion to application rates, by assuming that estimated ECx correspond to hypothetical predicted environmental concentrations (PECs) of bentazone and terbuthylazine in surface water and soil.

	Bentazone		Terbuthylazine		
	ECx (PECs)	Application rates (g ha^{-1})	ECx (PECs)	Application rates (g ha^{-1})	
<i>Raphidocelis subcapitata</i> ($\mu\text{g L}^{-1}$)	EC ₁	242.124	734.600	1.221	4.259
	EC ₅	680.882	2065.783	3.891	13.572
	EC ₁₀	1036.690	3145.297	6.23	21.741
	EC ₂₀	1695.313	5142.597	10.815	37.722
	EC ₅₀	3930.097	11923.838	27.749	96.788
<i>Brassica napus</i> (mg Kg^{-1} D.W.)	EC ₁	0.327	43.600	0.039	5.200
	EC ₅	0.643	85.733	0.115	15.333
	EC ₁₀	0.846	112.800	0.179	23.867
	EC ₂₀	1.167	155.600	0.298	39.733
	EC ₅₀	2.021	269.467	0.716	95.467
Dose recommended in Asteca® Mays	4.500	600.000	4.500	600.000	

References

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