

## Supporting Material

**Article title:** Age-specific allocation of plant chemical defenses within reproductive tissues: a test of the optimal defense theory

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The following Supporting Material is available for this article:

**Fig. S1a,b** Amount of glucosinolates by reproductive tissue according to the ontogenetic stage.

**Fig. S2a,b** Amount of glucosinolates in vegetative, accessory, and primary reproductive tissues.

**Fig. S3a,b** Amount of glucosinolates by ontogenetic stage according to the reproductive tissue.

**Fig. S4a,b** Amount of glucosinolates in vegetative and reproductive tissues, with reproductive tissues analyzed at three maturity stages for each treatment (constitutive vs. induced).

**Table S1** List of identified glucosinolates.

**Table S2** Results of ANOVAs testing the effect of the reproductive tissue, the maturity stage and their interaction on the constitutive amount of each glucosinolate.

**Table S3** Results of pairwise comparisons performed on the constitutive amount of each glucosinolate.

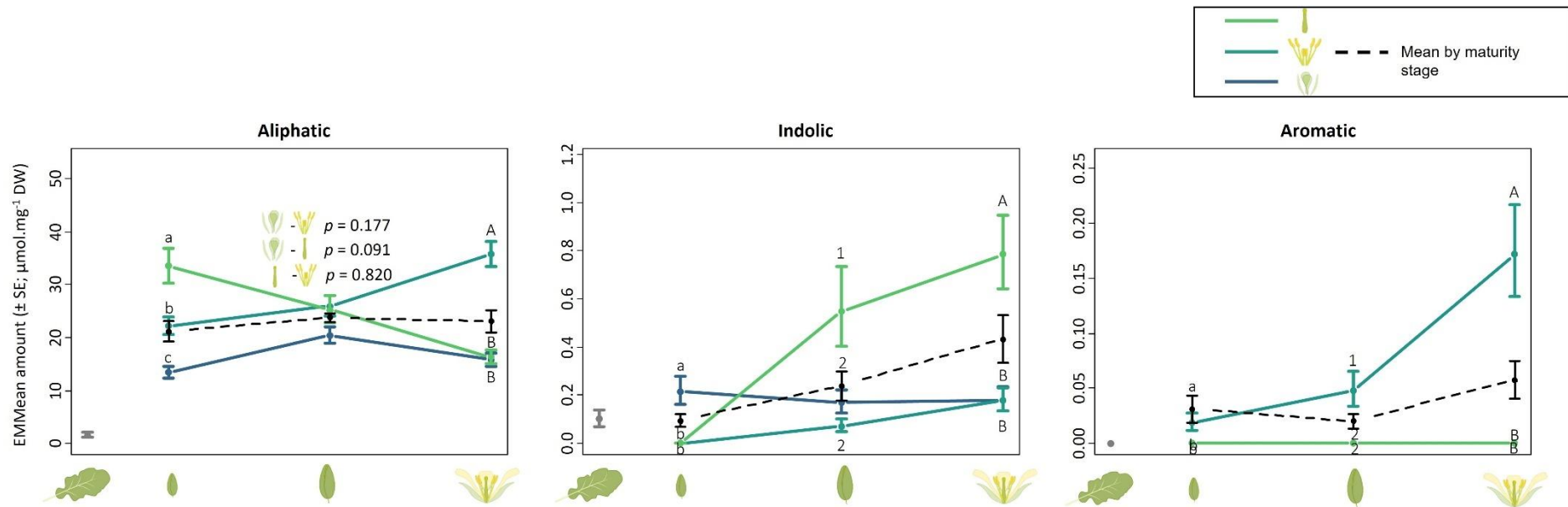


Figure S1.a: EMMean ( $\pm$  SE) amount of glucosinolate categories (aliphatic: glucoraphanin, progoitrin, glucoalyssin, gluconapoleiferin, gluconapin, glucobrassicinapin, glucoerucin, n-hexyl-GSL iso.I, n-hexyl-GSL iso.II, n-hexyl-GSL iso.III; indolic: glucobrassicin; aromatic: gluconasturtiin) in vegetative and reproductive tissues (youngest leaf vs. perianths, anthers and pistils), with reproductive tissues analyzed at three maturity stages (intermediate flower buds, old buds and flowers). Letters and numbers indicate significant differences between reproductive tissues for each maturity stage.  $n = 8$  per treatment with 10 plants per replicate, except for pistil of intermediate and old buds where  $n = 4$  with 20 plants per replicate.

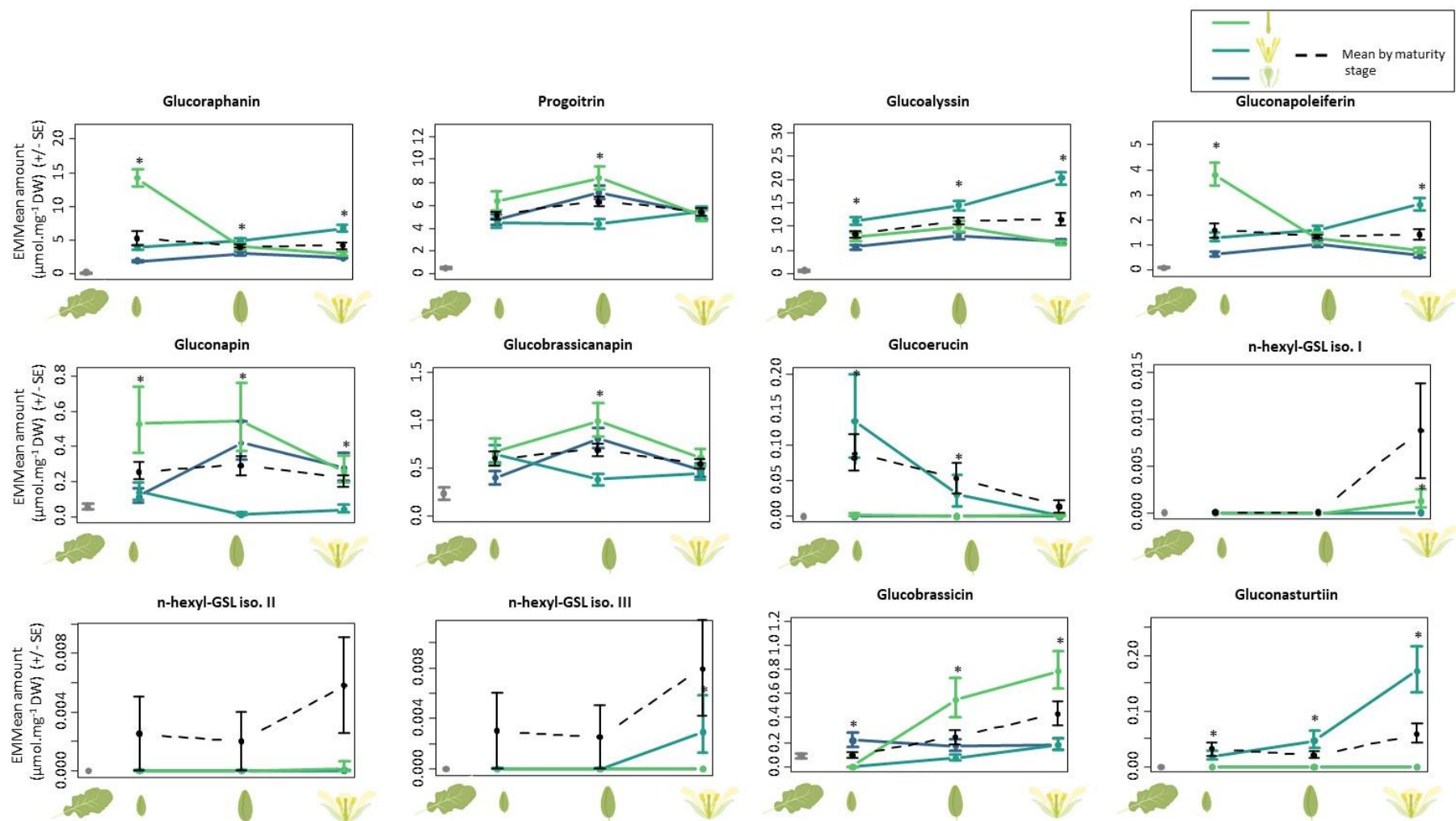


Figure S1.b: EM Mean ( $\pm$  SE) individual amount of constitutive glucosinolates in vegetative and reproductive tissues (youngest leaf vs. perianths, anthers and pistils), with reproductive tissues analyzed at three maturity stages (intermediate flower buds, old buds and flowers).  $n = 8$  per treatment with 10 plants per replicate, except for pistil of intermediate and old buds where  $n = 4$  with 20 plants per replicate. Asterisks indicate significant differences between at least two tissues for a given maturity stage (details in Table S3).

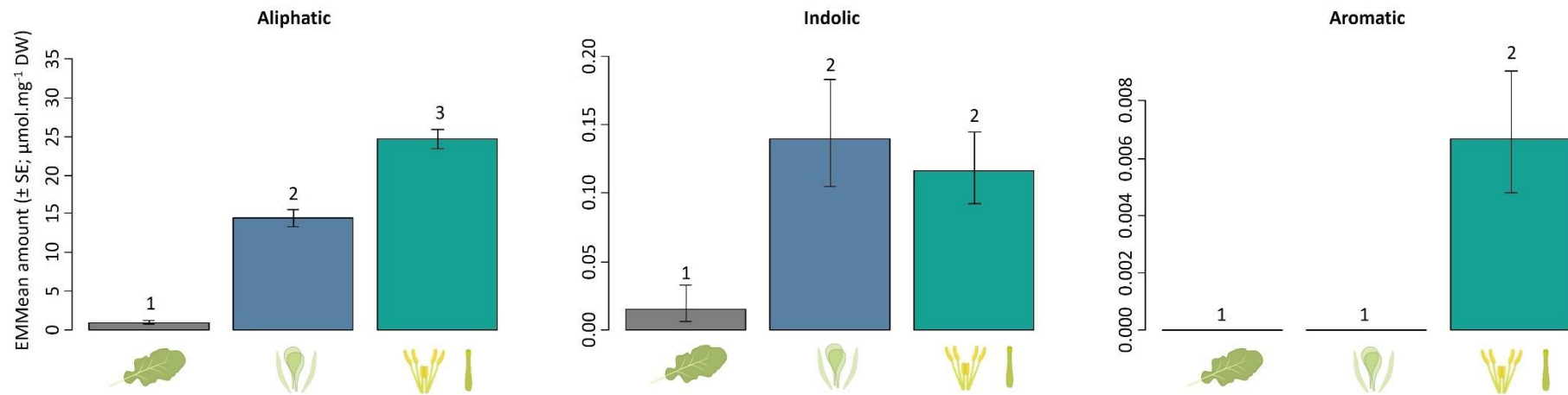


Figure S2.a: EMMean ( $\pm$  SE) amount of glucosinolate categories (aliphatic: glucoraphanin, progoitrin, glucoalyssin, gluconapoleiferin, gluconapin, glucobrassicinapin, glucoerucin, n-hexyl-GSL iso.I, n-hexyl-GSL iso.II, n-hexyl-GSL iso.III; indolic: glucobrassicin; aromatic: gluconasturtiin) in vegetative, accessory, and primary reproductive tissues (youngest leaf vs. perianths vs. anthers and pistils).  $n = 8$  for vegetative tissues with 10 plants per replicate,  $n = 24$  perianths with 10 plants per replicate,  $n = 40$  for primary reproductive tissues with 10 plants per replicate except for pistils of intermediate and old buds where there are 20 plants per replicate.

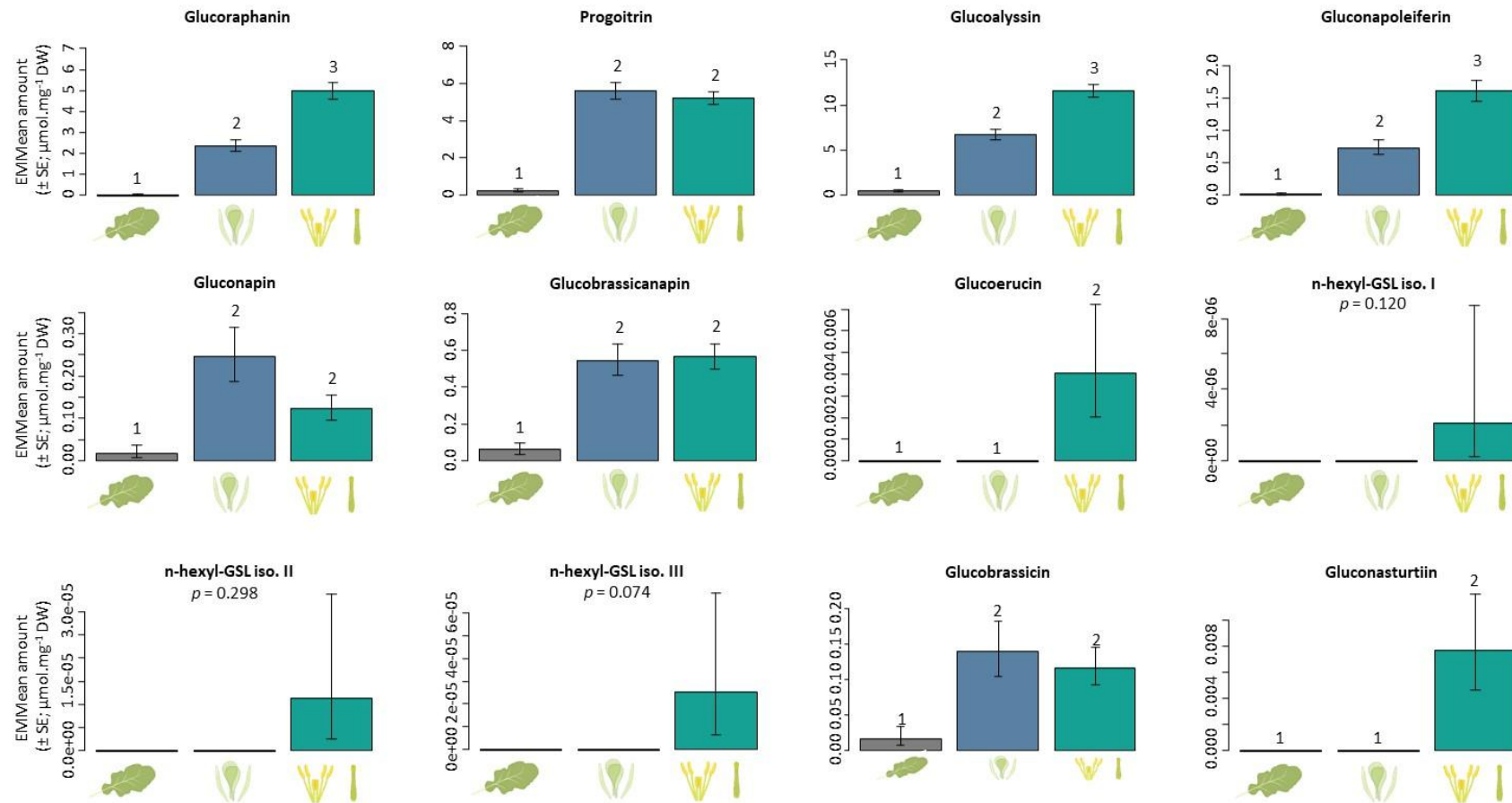


Figure S2.b: EM Mean (± SE) individual amount of constitutive glucosinolates in vegetative, accessory, and primary reproductive tissues (young

gest leaf vs. perianths vs. anthers and pistils).  $n = 8$  for vegetative tissues with 10 plants per replicate,  $n = 24$  perianths with 10 plants per replicate,  $n = 40$  for primary reproductive tissues with 10 plants per replicate except for pistils of intermediate and old buds where there are 20 plants per replicate.

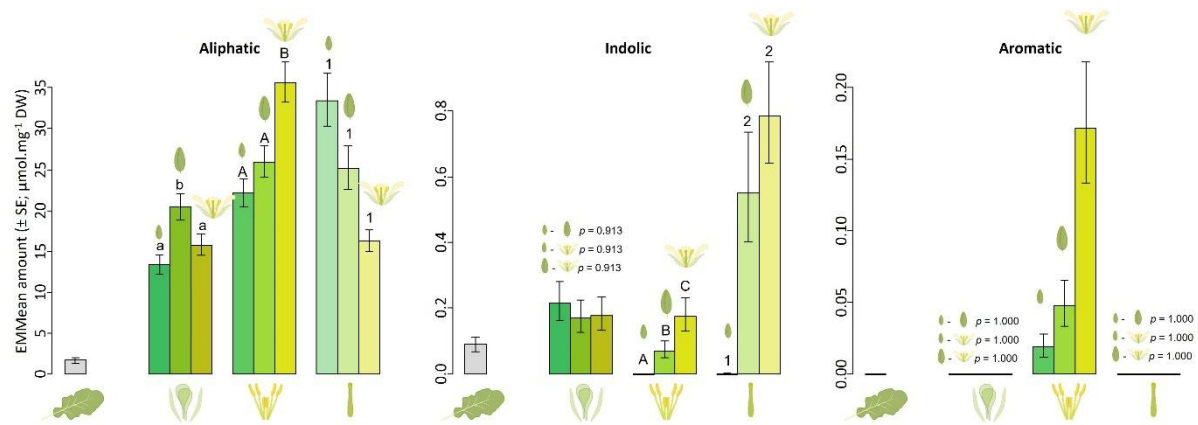


Figure S3.a: EMMean ( $\pm$  SE) amount of glucosinolate categories (aliphatic: glucoraphanin, progoitrin, glucoalyssin, gluconapoleiferin, gluconapin, glucobrassicinapin, glucoerucin, n-hexyl-GSL iso.I, n-hexyl-GSL iso.II, n-hexyl-GSL iso.III; indolic: glucobrassicin; aromatic: gluconasturtiin) in vegetative and reproductive tissues (youngest leaf vs. perianths, anthers and pistils), with reproductive tissues analyzed at three maturity stages (intermediate flower buds, old buds and flowers). Letters and numbers indicate significant differences between maturity stages for each reproductive tissue.  $n = 8$  per treatment with 10 plants per replicate, except for pistils of intermediate and old buds where  $n = 4$  with 20 plants per replicate.

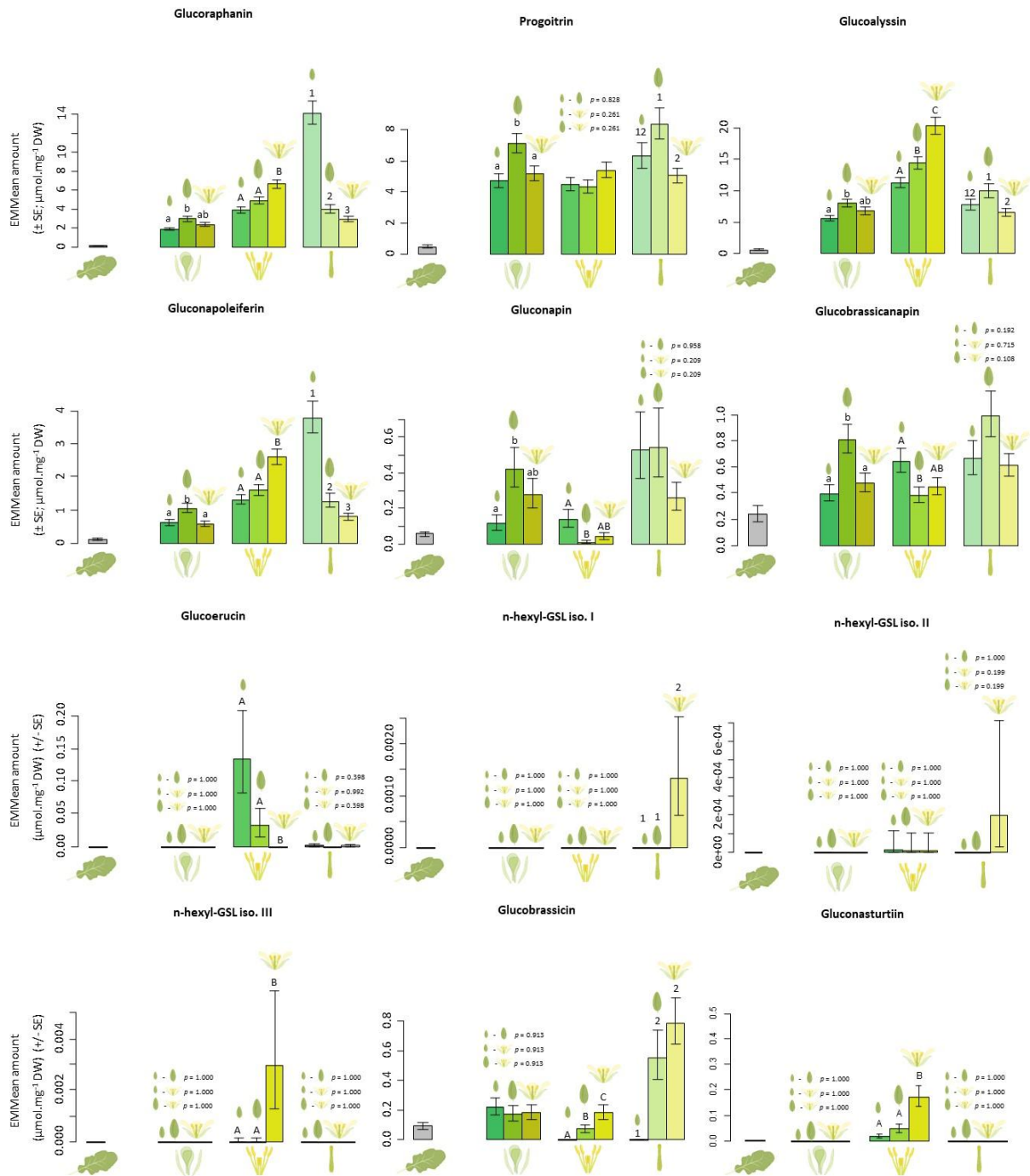


Figure S3.b: EMN/mean ( $\pm$  SE) individual amount of constitutive glucosinolates in vegetative and reproductive tissues (youngest leaf vs. perianths, anthers and pistils), with reproductive tissues analyzed at three maturity stages (intermediate flower buds, old buds and flowers). Letters and numbers indicate significant differences between maturity stages for each reproductive tissue.  $n = 8$  per treatment with 10 plants per replicate, except for pistils of intermediate and old buds where  $n = 4$  with 20 plants per replicate.

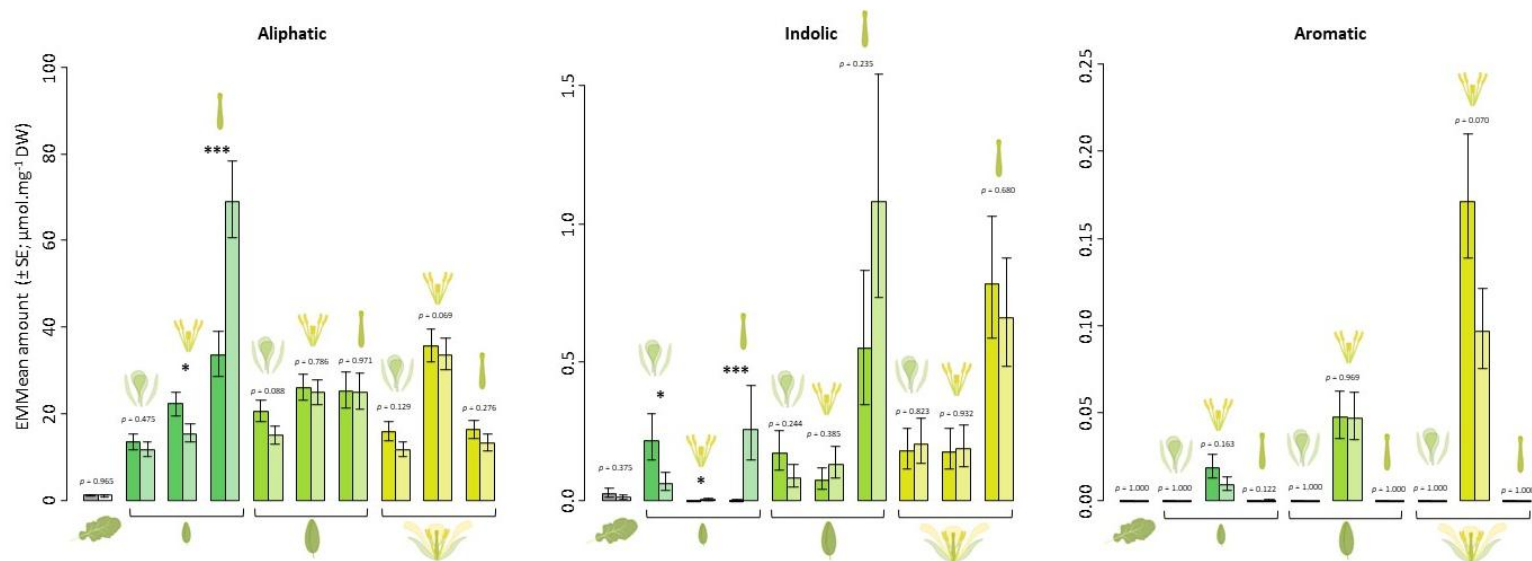


Figure S4.a: EM Mean ( $\pm$  SE) amount of glucosinolate categories (aliphatic: glucoraphanin, progoitrin, glucoalyssin, gluconapoleiferin, gluconapin, glucobrassicinapin, glucoerucin, n-hexyl-GSL iso.I, n-hexyl-GSL iso.II, n-hexyl-GSL iso.III; indolic: glucobrassicin; aromatic: gluconasturtiin) in vegetative and reproductive tissues (youngest leaf vs. perianths, anthers and pistils), with reproductive tissues analyzed at three maturity stages (intermediate flower buds vs. old buds and flowers) for each treatment (constitutive vs. induced). Asterisks indicate significant differences (\* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ ).  $n = 8$  per treatment with 10 plants per replicate, except for pistils of intermediate and old buds where  $n = 4$  with 20 plants per replicate.



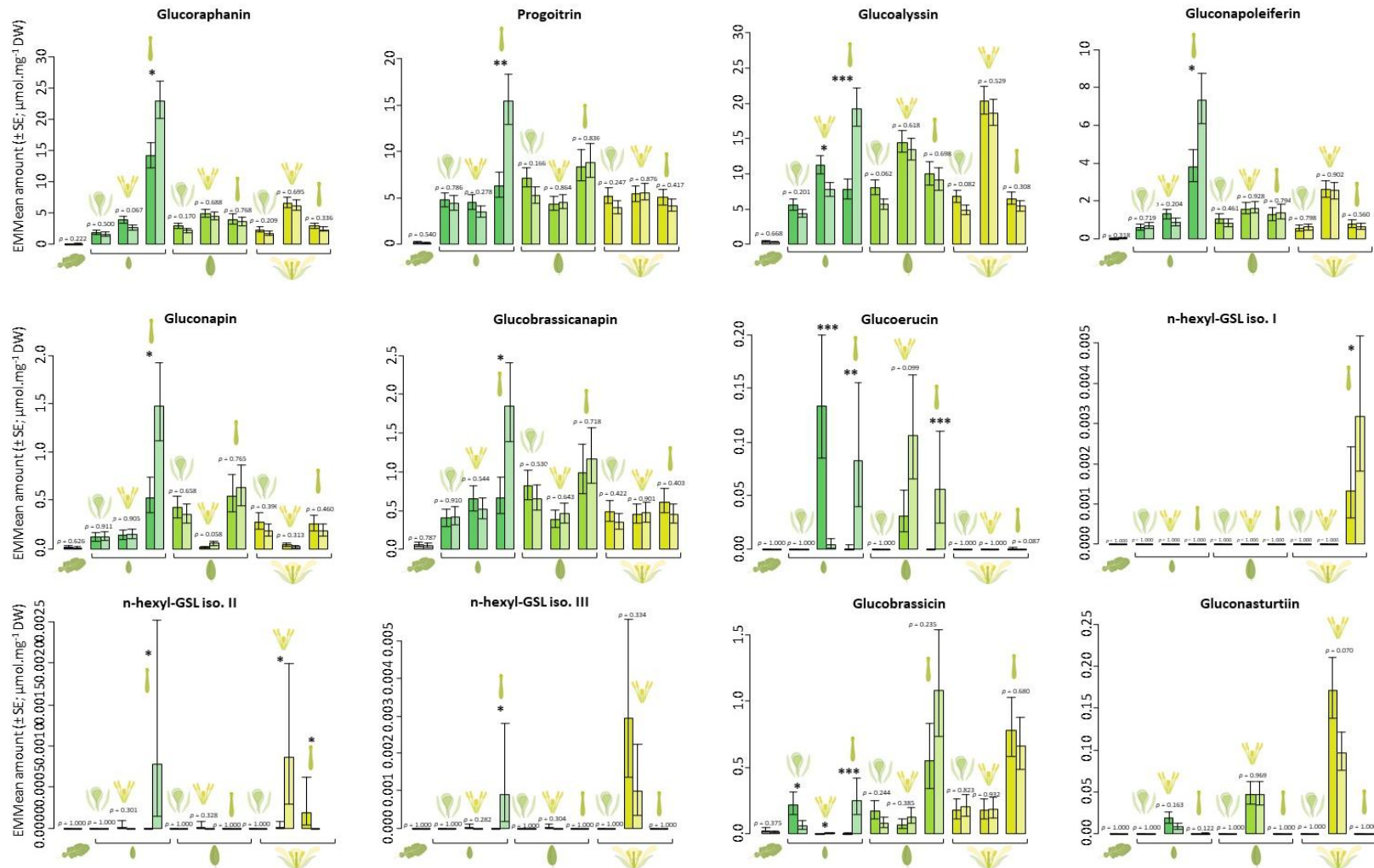


Figure S4.b: EM Mean ( $\pm$  SE) individual glucosinolate amount in vegetative and reproductive tissues (youngest leaf vs. perianths, anthers and pistils), with reproductive tissues analyzed at three maturity stages (intermediate flower buds vs. old buds and flowers) for each treatment (constitutive vs. induced). Asterisks indicate significant differences (\* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ ).  $n = 8$  per treatment with 10 plants per replicate, except for pistils of intermediate and old buds where  $n = 4$  with 20 plants per replicate.

Table S1: List of identified glucosinolates (GSL).

<b>m/z</b>	<b>RT (min)</b>	<b>Semi-systemic name</b>	<b>Trivial name</b>	<b>Category</b>	<b>Standard used for quantification</b>
436.04	3.18	4-methylsulfinylbutyl-GSL	Glucoraphanin	aliphatic	Glucoraphanin
388.04	3.4	2-hydroxy-3-butenyl-GSL	Progoitrin	aliphatic	Progoitrin
450.06	3.98	5-methylsulfinylpentyl-GSL	Glucoalyssin	aliphatic	Glucoalyssin
402.05	4.66	hydroxy-pentenyl-GSL	Gluconapoleiferin	aliphatic	Glucobrassicinapin
372.08	5.52	3-butenyl-GSL	Gluconapin	aliphatic	Gluconapin
386.06	8.36	4-pentenyl-GSL	Glucobrassicinapin	aliphatic	Glucobrassicinapin
420.05	9.17	4-methylthiobutyl-GSL	Glucoerucin	aliphatic	Glucoerucin
402.09	14.83	n-hexyl-GSL iso. I	-	aliphatic	Glucobrassicinapin
402.09	15.14	n-hexyl-GSL iso. II	-	aliphatic	Glucobrassicinapin
416.10	19.3	n-heptyl-GSL iso. III	-	aliphatic	Glucobrassicinapin
447.05	11.5	3-indolylmethyl-GSL	Glucobrassicin	indolic	Glucobrassicin
422.06	13.15	2-phenylethyl-GSL	Gluconasturtiin	aromatic	Gluconasturtiin

Table S2: Results of ANOVAs testing the effect of the reproductive tissue, the maturity stage and their interaction on the constitutive amount of each glucosinolate. Significant p-values are indicated in bold.  $n = 8$  per treatment with 10 plants per replicate, except for pistils of intermediate and old buds where  $n = 4$  with 20 plants per replicate.

	Aliphatic																	
	Glucoraphanin			Progoitrin			Glucoalyssin			Gluconapoleiferin			Gluconapin			Glucobrassicinapin		
	<i>F</i>	df1,df2	<i>p</i>	<i>F</i>	df1,df2	<i>p</i>	<i>F</i>	df1,df2	<i>p</i>	<i>F</i>	df1,df2	<i>p</i>	<i>F</i>	df1,df2	<i>p</i>	<i>F</i>	df1,df2	<i>p</i>
reproductive tissue	74.188	2,55	<0.001	5.237	2,55	<0.01	95.032	2,55	<0.001	43.792	2,55	<0.001	26.097	2,55	<0.001	5.292	2,55	<0.01
maturity stage	2.114	2,55	0.130	3.982	2,55	<0.05	10.941	2,55	<0.001	1.774	2,55	0.179	0.491	2,55	0.615	2.540	2,55	0.088
reproductive tissue:maturity stage	48.562	4,55	<0.001	4.293	4,55	<0.01	8.578	4,55	<0.001	26.031	4,55	<0.001	6.292	4,55	<0.001	5.103	4,55	<0.01

	Aliphatic												Indolic			Aromatic		
	Glucoerucin			n-hexyl-GSL iso. I			n-hexyl-GSL iso. II			n-hexyl-GSL iso. III			Glucobrassicin			Gluconasturtiin		
	<i>F</i>	df1,df2	<i>p</i>	<i>F</i>	df1,df2	<i>p</i>	<i>F</i>	df1,df2	<i>p</i>	<i>F</i>	df1,df2	<i>p</i>	<i>F</i>	df1,df2	<i>p</i>	<i>F</i>	df1,df2	<i>p</i>
reproductive tissue	17.820	2,55	<0.001	4.941	2,55	<0.05	1.451	2,55	0.243	7.269	2,55	<0.01	34.367	2,55	<0.001	150.959	2,55	<0.001
maturity stage	6.468	2,55	<0.01	2.223	2,55	0.118	0.459	2,55	0.634	1.874	2,55	0.163	62.287	2,55	<0.001	4.622	2,55	<0.05
reproductive tissue:maturity stage	6.960	4,55	<0.001	2.964	4,55	<0.05	0.644	4,55	0.634	1.639	4,55	0.177	23.951	4,55	<0.001	3.967	4,55	<0.01

Table S3: Results of pairwise comparisons performed on the constitutive amount of each glucosinolates. Comparisons are performed between reproductive tissues (perianths, anthers and pistils) for each of the three maturity stages (intermediate flower buds, old buds and flowers). Significant *p*-values are indicated in bold. *n* = 8 per treatment with 10 plants per replicate, except for pistils of intermediate and old buds where *n* = 4 with 20 plants per replicate.

		Aliphatic																	
		Glucoraphanin			Progoitrin			Glucoalyssin			Gluconapoleiferin			Gluconapin			Gluco Brassicanapin		
		<i>t</i>	df	<i>p</i>	<i>t</i>	df	<i>p</i>	<i>t</i>	df	<i>p</i>	<i>t</i>	df	<i>p</i>	<i>t</i>	df	<i>p</i>	<i>t</i>	df	<i>p</i>
Intermediate bud	perianth - anther	5.709	55	<0.001	0.369	55	0.713	6.091	55	<0.001	4.094	55	<0.001	0.392	55	0.697	2.371	55	0.064
	perianth - pistil	15.304	55	<0.001	1.822	55	0.111	2.237	55	0.029	9.486	55	<0.001	2.944	55	<0.05	2.068	55	0.065
	anther - pistil	10.643	55	<0.001	2.123	55	0.111	2.737	55	0.012	6.144	55	<0.001	2.624	55	<0.05	0.132	55	0.895
Old bud	perianth - anther	4.227	55	<0.001	3.783	55	<0.001	5.610	55	<0.001	2.527	55	0.043	6.248	55	<0.001	3.750	55	<0.001
	perianth - pistil	1.973	55	0.080	1.080	55	0.285	1.652	55	0.104	0.904	55	0.370	0.571	55	0.571	0.930	55	0.356
	anther - pistil	1.479	55	0.145	4.169	55	<0.001	2.929	55	<0.01	1.159	55	0.370	5.672	55	<0.001	3.992	55	<0.001
Flower	perianth - anther	8.950	55	<0.001	0.291	55	0.828	10.611	55	<0.001	9.163	55	<0.001	3.658	55	<0.01	0.353	55	0.725
	perianth - pistil	1.734	55	0.088	0.219	55	0.828	0.349	55	0.728	1.670	55	0.100	0.141	55	0.888	1.183	55	0.363
	anther - pistil	7.216	55	<0.001	0.510	55	0.828	10.960	55	<0.001	7.492	55	<0.001	3.517	55	<0.01	1.536	55	0.363

		Aliphatic												Indolic			Aromatic		
		Glucorucin			n-hexyl-GSL iso. I			n-hexyl-GSL iso. II			n-hexyl-GSL iso. III			Glucobrassicin			Gluconasturtiin		
		<i>t</i>	df	<i>p</i>	<i>t</i>	df	<i>p</i>	<i>t</i>	df	<i>p</i>	<i>t</i>	df	<i>p</i>	<i>t</i>	df	<i>p</i>	<i>t</i>	df	<i>p</i>
Intermediate bud	perianth - anther	6.085	55	<0.001	0.000	55	1.000	0.934	55	0.673	0.998	55	0.628	10.526	55	<0.001	4.094	55	<0.001
	perianth - pistil	1.300	55	0.199	0.000	55	1.000	0.000	55	1.000	0.000	55	1.000	6.822	55	<0.001	9.486	55	<0.001
	anther - pistil	3.668	55	<0.001	0.000	55	1.000	0.763	55	0.673	0.815	55	0.628	1.773	55	0.082	6.144	55	<0.001
Old bud	perianth - anther	4.228	55	<0.001	0.000	55	1.000	0.883	55	0.711	0.954	55	0.659	1.940	55	0.057	2.527	55	0.043
	perianth - pistil	0.000	55	1.000	0.000	55	1.000	0.000	55	1.000	0.000	55	1.000	2.763	55	<0.05	0.904	55	0.37
	anther - pistil	3.452	55	<0.01	0.000	55	1.000	0.721	55	0.711	0.779	55	0.659	4.348	55	<0.001	1.159	55	0.37
Flower	perianth - anther	0.000	55	1.000	0.000	55	1.000	0.883	55	0.381	3.756	55	<0.001	0.011	55	0.991	9.163	55	<0.001
	perianth - pistil	1.579	55	0.180	4.038	55	<0.001	1.868	55	0.201	0.000	55	1.000	4.508	55	<0.001	1.67	55	0.101
	anther - pistil	1.579	55	0.180	4.038	55	<0.001	0.985	55	0.381	3.756	55	<0.001	4.519	55	<0.001	7.492	55	<0.001