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Supplementary materials

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Table S1: Herbicides and doses used in the screening of experimentally derived non-target-site resistant blackgrass seed populations. The first column ('Resistance') denotes the herbicide mode-of-action to which NTSR resistance has been evolved. 'Herbicide' refers to the herbicide applied under glasshouse conditions, while 'Line' denotes the separate paired 'S' and 'R' seed lines studied. Doses are given as grams of the active ingredient per hectare. The herbicide "Atlantis" represents a commercial formulation of the sulfonylurea actives mesosulfuron-methyl and iodosulfuron-methyl-sodium.

Resistance HRAC	Herbicide	Line	Dose (g ai ha ⁻¹)										
Group 1: ACCase, (segregating line from R x S pairwise cross)	Fenoxaprop	"S"	0	1.08	2.16	4.31	8.63	17.3	34.5	69	138	276	552
		"R"	0	4.31	8.63	17.3	34.5	69	138	276	552	1104	1656
	Cinmethylin	"S"	0	3.9	7.8	15.6	31.3	62.5	125	250	500	1000	2000
		"R"	0	3.9	7.8	15.6	31.3	62.5	125	250	500	1000	2000
Group 2: ALS, (selected with Atlantis for two generations)	Atlantis	"S"	0	0.23	0.45	0.9	1.8	3.6	7.2	14.4	28.8	57.6	115.2
		"R"	0	0.9	1.8	3.6	7.2	14.4	28.8	57.6	115.2	230.4	345.6
	Cinmethylin	"S"	0	3.9	7.8	15.6	31.3	62.5	125	250	500	1000	2000
		"R"	0	3.9	7.8	15.6	31.3	62.5	125	250	500	1000	2000
Group 3: Microtubule assembly, (selected with Pendimethalin for nine generations)	Pendimethalin	"S"	0	18.8	37.5	75	150	300	600	1200	2400	4800	9600
		"R"	0	37.5	75	150	300	600	1200	2400	4800	9600	14400
	Cinmethylin	"S"	0	3.9	7.8	15.6	31.3	62.5	125	250	500	1000	2000
		"R"	0	3.9	7.8	15.6	31.3	62.5	125	250	500	1000	2000
Group 15: VLCFA, (selected with flufenacet for nine generations)	Flufenacet	"S"	0	3.75	7.5	15	30	60	120	240	480	960	1920
		"R"	0	3.75	7.5	15	30	60	120	240	480	960	1920
	Cinmethylin	"S"	0	3.9	7.8	15.6	31.3	62.5	125	250	500	1000	2000
		"R"	0	3.9	7.8	15.6	31.3	62.5	125	250	500	1000	2000

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10 **Table S2: Percentage seedling emergence, and percentage dry weight of a single emerged seedling from**
 11 **the glasshouse assay. Results are the mean (\pm standard deviation) calculated from all field collected**
 12 **'Lola' populations combined.**

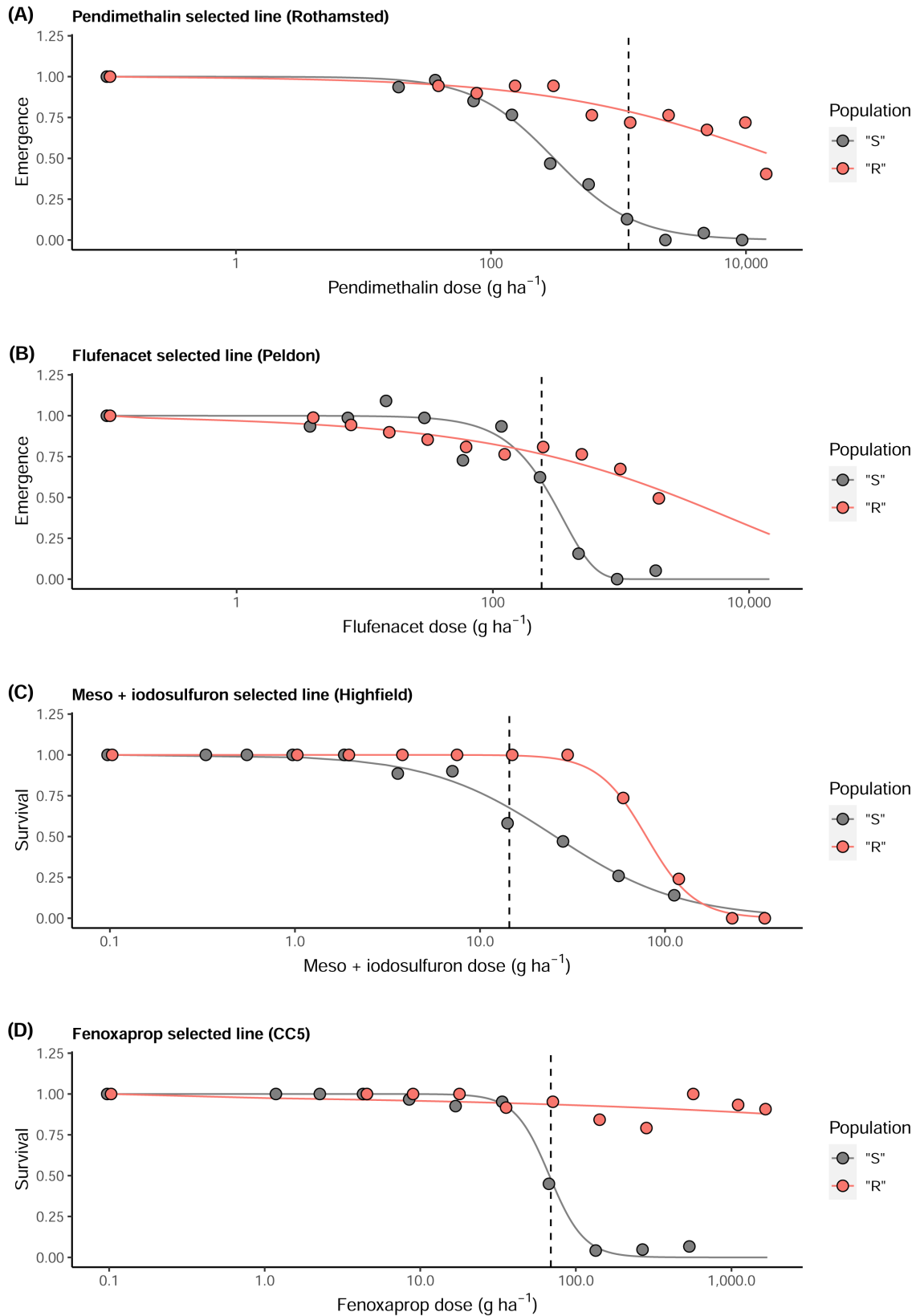
Herbicide	Dose (g ai ha⁻¹)	Percentage seedling emergence (\pm St.dev)		Percentage weight of a single plant (\pm St.dev)	
cinmethylin	0	100	(\pm 12.2)	100	(\pm 34)
cinmethylin	15	59.1	(\pm 30.3)	41.6	(\pm 28)
cinmethylin	50	25.9	(\pm 26.6)	26.0	(\pm 24.3)
cinmethylin	125	9.18	(\pm 19.2)	25.6	(\pm 28.2)
cinmethylin	250	4.87	(\pm 13)	22.7	(\pm 16.3)
cinmethylin	500	2.3	(\pm 8.17)	17.4	(\pm 16.7)

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17 **Table S3: Percentage seedling emergence from the outdoor container assay. Results are the mean (\pm**
 18 **standard deviation) calculated from the two field collected 'Lola' populations included: 'Lola 8', and 'Lola**
 19 **91'.**

Herbicide	Dose (g ai ha⁻¹)	Percentage seedling emergence (\pm St.dev)	
cinmethylin	0	100	(\pm 8.43)
cinmethylin	31.2	56.6	(\pm 35)
cinmethylin	62.5	31.1	(\pm 28.2)
cinmethylin	125	23.4	(\pm 20.7)
cinmethylin	250	11.5	(\pm 9.73)
cinmethylin	500	2.24	(\pm 2.07)
flufenacet	180	2.78	(\pm 2.06)
pendimethalin	1200	73.5	(\pm 19)

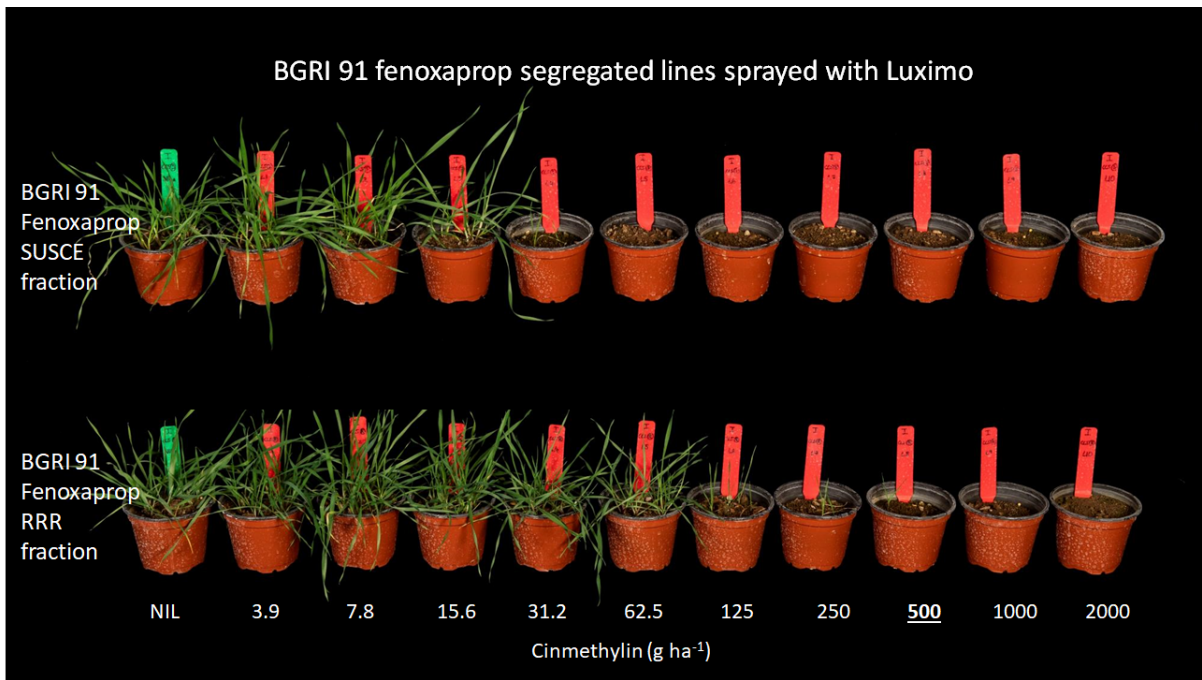
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Figure S1: Confirmation of resistance to selected herbicides within experimental resistance lines. In each case the "R" and "S" lines are derived from the same genetic background, but have been experimentally selected for differences in non-target-site resistance to a particular herbicide. Lines were screened under glasshouse conditions for differences in resistance to their selected herbicide: (A) Pendimethalin, (B)

26 Flufenacet, (C) Meso+Iodosulfuron, (D) Fenoxaprop. For herbicides applied pre-emergence
27 (Pendimethalin and Flufenacet), 'Emergence' was assessed as the number of healthy plants growing 5-6
28 weeks after exposure. For herbicides applied post-emergence (meso+iodosulfuron, fenoxaprop),
29 treatments were applied to growing plants at the three leaf stage, and plant survival assessed four weeks
30 later. In all cases, the vertical dashed line represents the UK field-rate herbicide dose.

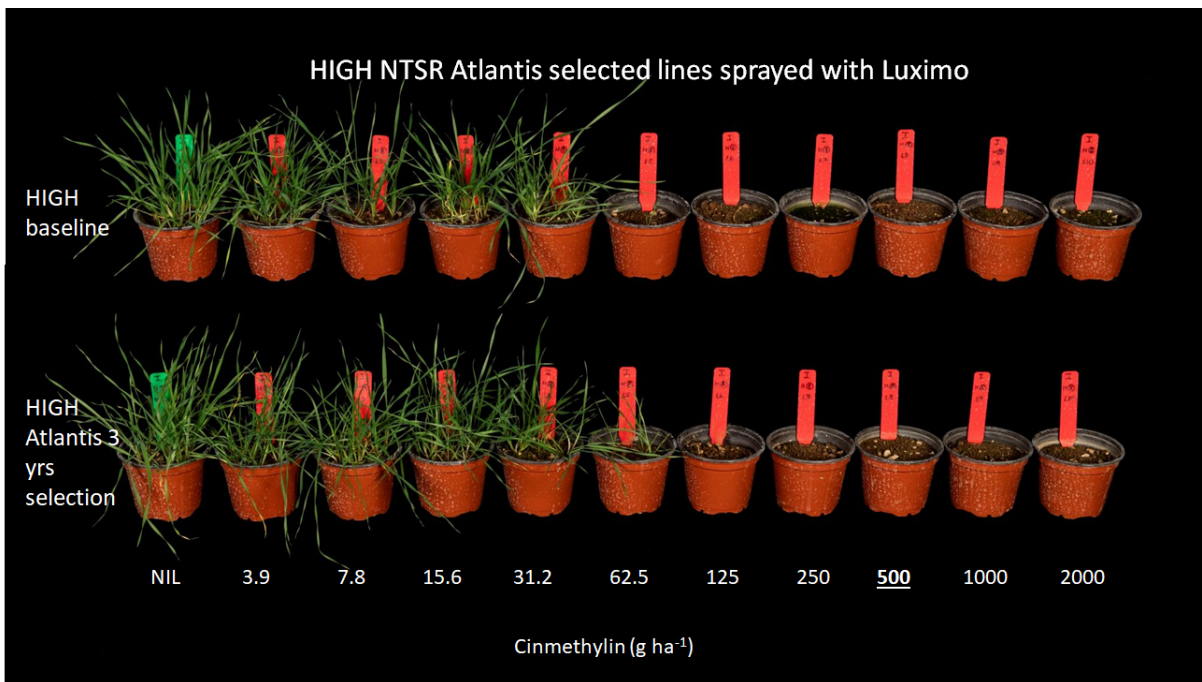


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32 **Figure S2: Representative images of the fenoxaprop resistant segregating line, treated with cinmethylin**
 33 **under glasshouse conditions. Cinmethylin was applied post-emergence.**

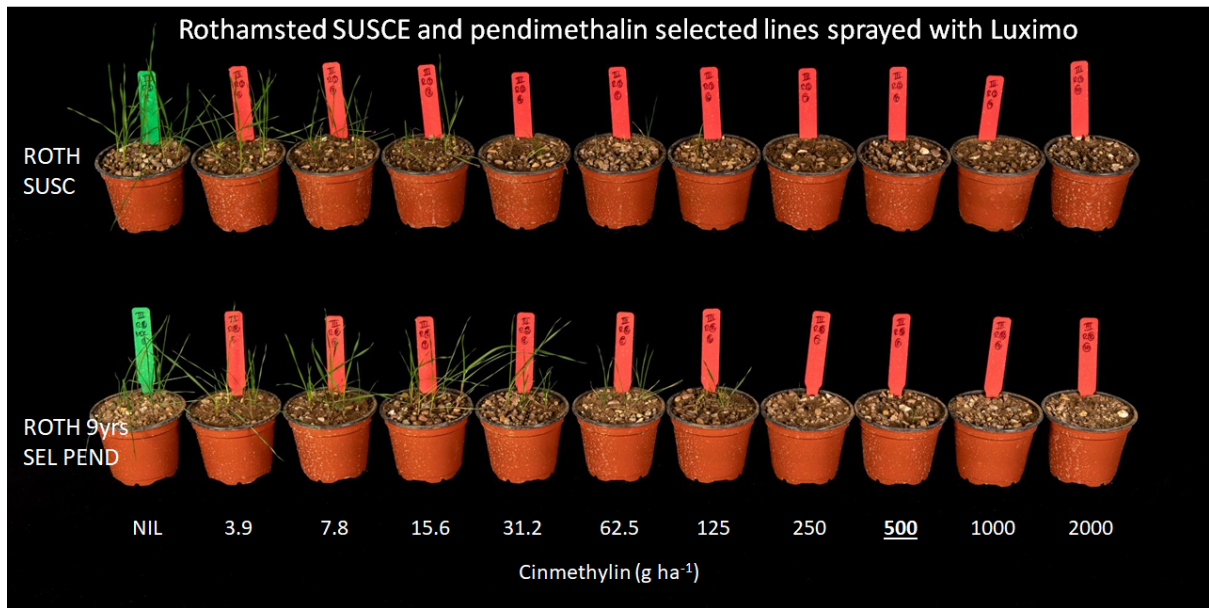
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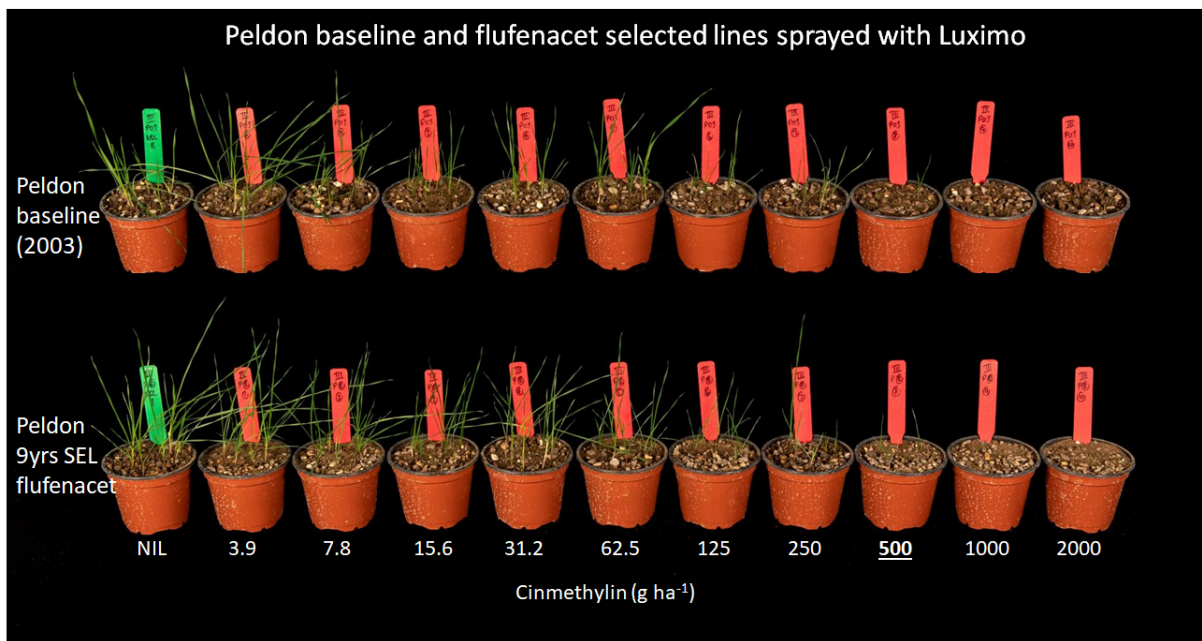
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37 **Figure S3: Representative images of the meso+iodosulfuron resistant segregating line, treated with**
 38 **cinmethylin under glasshouse conditions. Cinmethylin was applied post-emergence.**



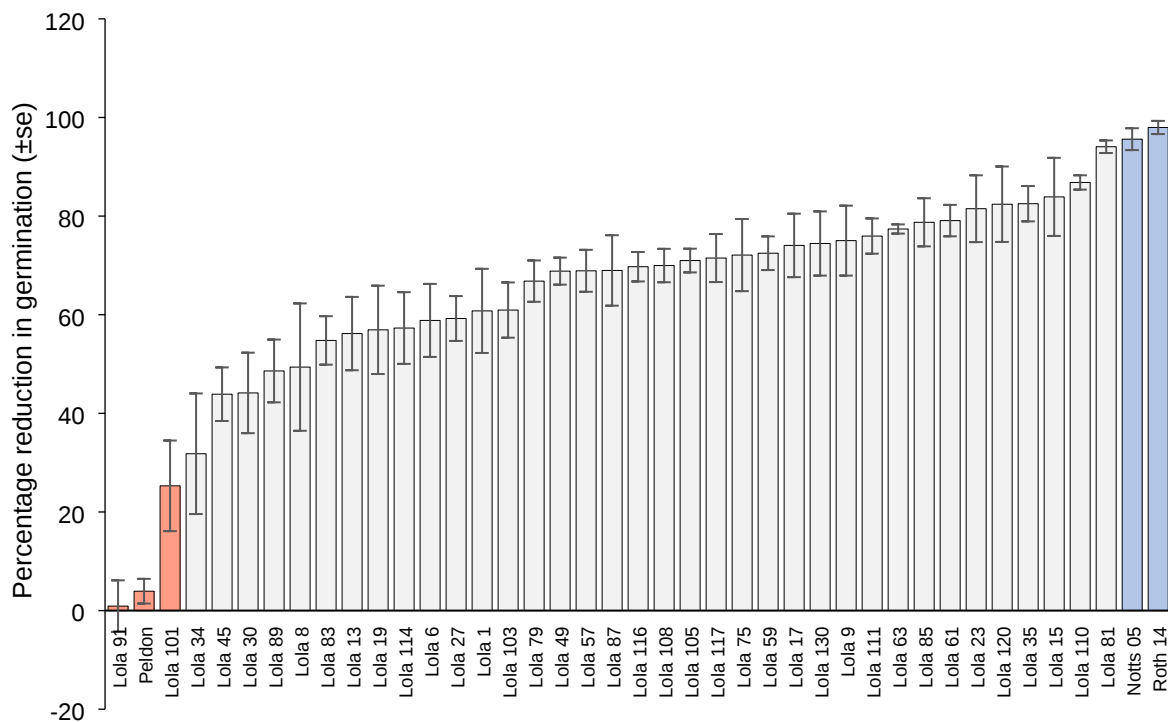
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Figure S4: Representative images of the pendimethalin resistant segregating line, treated with cinmethylin under glasshouse conditions. Cinmethylin was applied pre-emergence.



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Figure S5: Representative images of the flufenacet resistant segregating line, treated with cinmethylin under glasshouse conditions. Cinmethylin was applied pre-emergence.



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49 **Figure S6: Past population-level screening data for pendimethalin sensitivity. Populations are along the**
50 **X-axis, while the Y-axis gives the percentage reduction in seedling germination after pendimethalin**
51 **treatment. Populations found in the current study to have the lowest sensitivity to cinmethylin are**
52 **coloured red, while the sensitive standard populations with no NTSR and high sensitivity to cinmethylin**
53 **are highlighted in blue. Pendimethalin sensitivity was assayed at a single dose (5 ppm) using petri-**
54 **dishes, as described in (REF). Briefly, four control (untreated) and four pendimethalin treated dishes**
55 **were set up for each population, each testing 50 seeds. Dishes were kept within an incubator at 17/11 °C**
56 **over 14/10 hour cycles for two weeks. After this time, the number of healthy, germinated seeds was**
57 **counted in both treated and control dishes, and used to calculate a percentage reduction in germination**
58 **caused by the herbicide.**