A Non-destructive Leaf Disc Assay for Rapid Diagnosis of Weed Resistance to Multiple Herbicides

Chenxi Wu, Vijaya Varanasi, and Alejandro Perez-Jones

Bayer CropScience, 700 Chesterfield Parkway W, St Louis, MO, 63017

Corresponding author: Chenxi Wu, chenxi.wu@bayer.com

Followings tables and figures are available as supporting information for the manuscript.

Table S1. Geographical origins of the weed populations

Table S2. Estimates of sample size needed for whole plant spray test and leaf disc assay to distinguish if a weed population is more resistant than another.

Table S3. Comparison of the results from both the leaf disc assays and the whole plant spray tests using Fv/Fm values relative to that of untreated leaf (%).

Figure S1. Leaf disc chlorophyll fluorescence images and photos of pigweed populations sprayed with glyphosate.

Figure S2. Leaf disc chlorophyll fluorescence images and photos of kochia plants sprayed with dicamba.

Figure S3. Regression lines showing the correlation between the Fv/Fm values and leaf area (expressed as a percentage of the untreated plants) with spray visual injuries.

Figure S4. Violin- and box-plots showing Fv/Fm, leaf area and spray injuries of different pigweed populations across different fomesafen doses.

Figure S5. Leaf disc chlorophyll fluorescence images and visual injuries of individual pigweed plants sprayed with fomesafen (Pop 1-12).

Figure S6. Leaf disc chlorophyll fluorescence images and visual injuries of individual pigweed plants sprayed with fomesafen (Pop 1-11).

Figure S7. Leaf disc chlorophyll fluorescence images and visual injuries of individual pigweed plants sprayed with fomesafen (Pop 9, 11 & 13-22).

Figure S8. Leaf disc chlorophyll fluorescence images and visual injuries of individual goosegrass plants sprayed with glyphosate.

Figure S9. Leaf disc chlorophyll fluorescence images and visual injuries of individual goosegrass plants sprayed with glyphosate at different evaluation time points.

Table S1 Geographical origins of the weed populations

Species	Pop	Id	Lat	Lon	Georgraphical origins
ELEIN	1	GR-Malaysia	N/A	N/A	Malaysia
ELEIN	2	WR2015-009-09	N/A	N/A	Malaysia
ELEIN	3	WR2015-010-24	N/A	N/A	Malaysia
ELEIN	4	WR2016-011	39.5824	-94.7264	14760 Kelley Rd SE, MO, USA
ELEIN	5	WT	N/A	N/A	Malaysia
AMAPA	1	IHX-2018-T1-118-2	35.63281	-89.46627	Durhamville, TN, USA (F1 lines)
AMATA	2	IHX-2018-T10-128	38.5962	-89.2495	Carlyle (Clinton County), IL, USA (F1 lines)
AMAPA	3	IHX-2018-T11-213	35.63281	-89.46627	Durhamville, TN, USA (F1 lines)
AMAPA	4	IHX-2018-T2-283-2	35.63281	-89.46627	Durhamville, TN, USA (F1 lines)
AMAPA	5	IHX-2018-T3-284	35.63281	-89.46627	Durhamville, TN, USA (F1 lines)
AMAPA	6	IHX-2018-T4-287	35.63281	-89.46627	Durhamville, TN, USA (F1 lines)
AMAPA	7	IHX-2018-T7-282	35.63281	-89.46627	Durhamville, TN, USA (F1 lines)
AMAPA	8	IHX-2018-T9-173-9	35.34639	-90.47722	Twist (Cross County), AR, USA (F1 lines)
AMAPA	9	WR2015-008	35.63281	-89.46627	Durhamville, TN, USA
AMATA	10	WR2013-013	38.76479	-88.9853	Kinmundy (Marion County), IL, USA
AMAPA	11	WR2016-010	38.5962	-89.2495	Carlyle (Clinton County), IL, USA
AMAPA	12	WR2016-027	33.47361	-90.47667	Moorhead (Sunflower County), MS USA
AMAPA	13	GS	N/A	N/A	Alfalfa county, northwest OK, USA
AMAPA	14	IHX-2018-T9-138-1	N/A	N/A	Olive Branch Desoto County, MS USA
AMAPA	15	WR2015-002	35.63281	-89.46627	Durhamville, TN, USA
AMAPA	16	WR2016-018	32.925	-90.89917	Rolling Fork (Sharkey County), MS, USA
AMAPA	17	WR2016-050	34.15417	-90.54111	Clarksdale (Coahoma County), MS, USA
AMAPA	18	WR2016-051	33.51111	-90.82889	Stoneville (Washington County), MS, USA
AMAPA	19	WR2016-053	33.40306	-90.62194	Indianola (Sunflower County), MS, USA
AMAPA	20	WR2016-057	35.34639	-90.47722	Twist (Cross County), AR, USA
AMAPA	21	WR2016-064	34.49833	-90.60083	Helena (Phillips County), AR, USA
AMATA	22	HGPL3	N/A	N/A	MO USA
AMATA	23	WR2013-009	41.72335	-93.54126	Ankeny (Polk County), IA USA

Table S2. Estimates of sample size needed for whole plant spray test and leaf disc assay to distinguish if a weed population is more resistant than another. We assume three scenarios with weed populations of different homogeneous levels.

Scenario	Population	Spray visual injury (%) of individual plant			Mean (μ)	SD	Pooled SD	Spray test sample size (N)[a]	Leaf disc assay sample size (N') based on accuracy		
		1	2	3					100%	75%	50%
1	1	90	90	100	93.3	5.8	15.9	1	1	1.3	2
	2	10	15	50	25.0	21.8					
2	1	90	90	100	93.3	5.8	11.5	8	8	10.7	16
	2	60	90	80	76.7	15.3					
3	1	60	70	100	76.7	20.8	29.5	41	41	54.7	82
	2	100	40	35	58.3	36.2					

[a]Sample sizes were calculated using the following online tool, with power (β)=0.8, α=0.05, 2-sided test: https://www.stat.ubc.ca/~rollin/stats/ssize/n2.html

In general, bigger sample sizes are needed to distinguish populations that segregates and similar in resistance levels. The sample size for leaf disc assay were just increased in proportion to accuracy levels.

Table S3. Comparison of the results from the leaf disc assays and the whole plant spray tests using Fv/Fm values expressed as the percentages of the untreated leaf (%).

Chemistry	Weed Species	Populations Tested (N)	Plants Tested (N)	Dose (µM)	LD Assay vs WPS [a]		
					Concordant [b]	False Positive	False Negative
fomesafen	*A. tuberculatus, A. palmeri*	23	**241**	***10*** [c]	**81.7%**	**14.9%**	**3.3%**
			232	*50*	77.2%	9.1%	13.8%
			241	*100*	65.6%	8.3%	26.1%
glyphosate	*E. indica*	5	**78**	***250***	**89.7%**	**9.0%**	**1.3%**
			77	*500*	75.3%	13.0%	11.7%
			74	*1000*	59.5%	21.6%	18.9%
glyphosate	*A. tuberculatus, A. palmeri*	4	45	*500*	71.1%	28.9%	0.0%
			36	*1000*	77.8%	13.9%	8.3%
			45	*2500*	53.3%	6.7%	40.0%
dicamba	*B. scoparia*	4	46	*250*	69.6%	30.4%	0.0%
			46	*500*	69.6%	30.4%	0.0%
			46	**800**	**67.4%**	**26.1%**	**6.5%**

[a] LD stands for the leaf disc assay, plants were considered resistant if Fv/Fm > 25%; WPS stands for whole plant spray test, plants were considered resistant if spray injury <90%.

[b] Results from the two methods were considered concordant when the same resistance calls were reported by both methods (e.g. a sprayed plant with <90% visual injury also has a Fv/Fm value > 25%).

[c] Highlighted are discriminating doses with the best regression relationship between chlorophyll fluorescence and herbicide spray injury data and lowest false positive/negative callings.

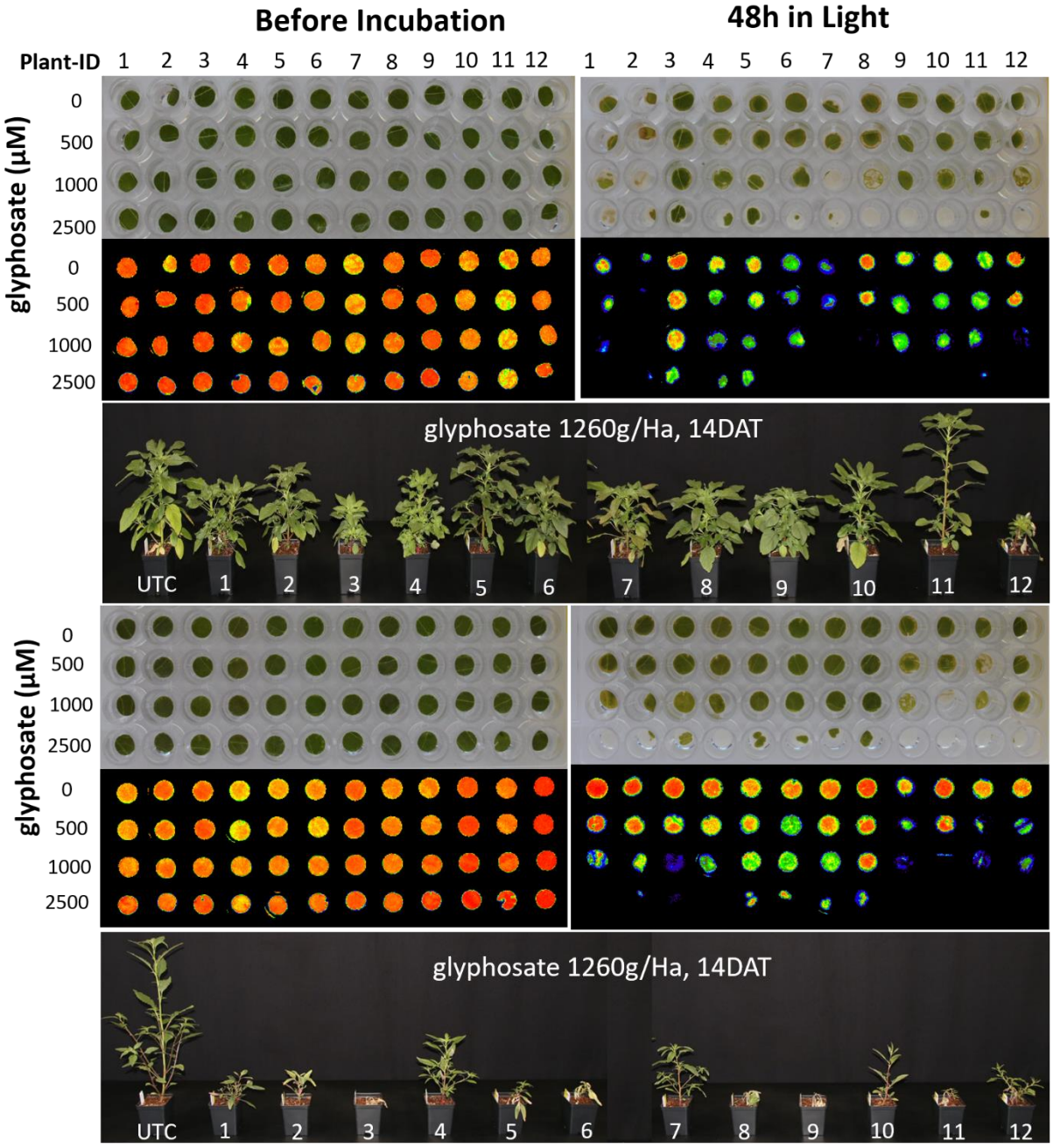

Figure S1. Leaf disc chlorophyll fluorescence images and photos of pigweed populations sprayed with glyphosate. The top panels are photos of the leaf discs taken before incubation and 36h after being incubated in herbicide solutions under light. Middle panels are corresponding chlorophyll images for the leaf discs in the top panel. Bottom panel are photos of sprayed plants from which the leaf discs were sampled from (4 leaf punches per plant). The top set of the plants were palmer amaranth and the bottom set of plants were common waterhemp.

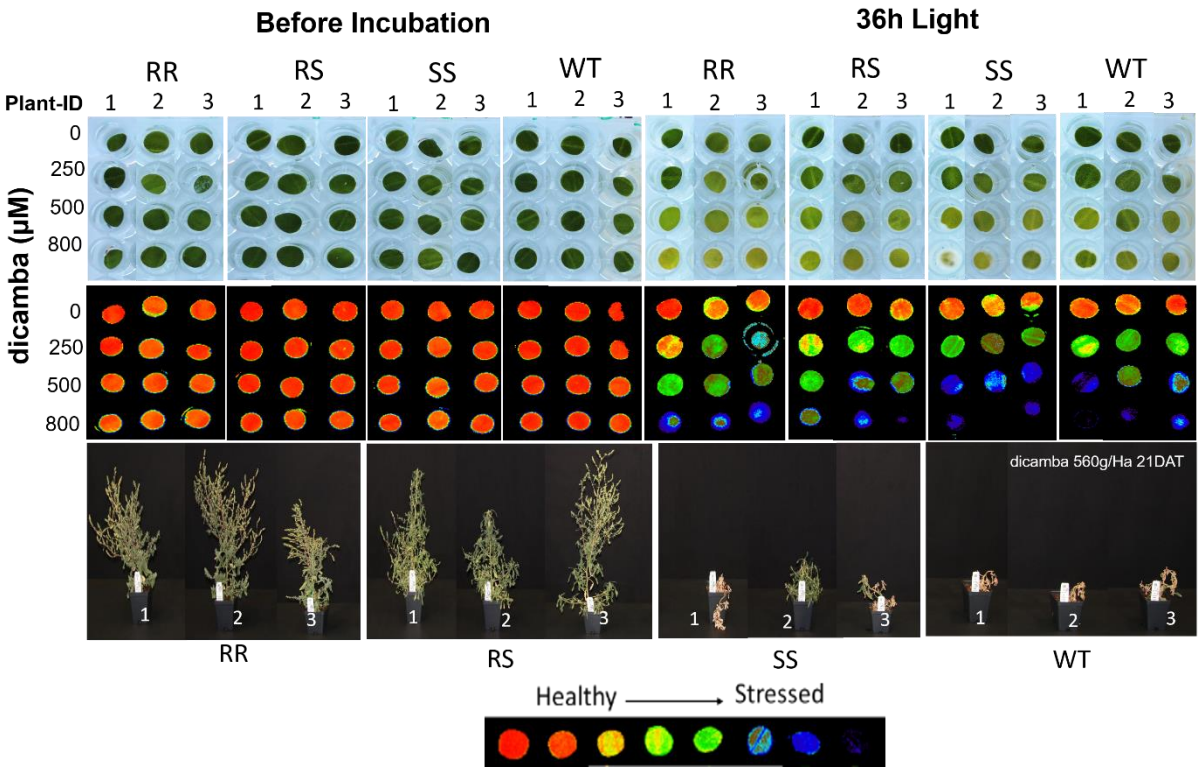

Figure S2. Leaf disc chlorophyll fluorescence images and photos of kochia plants sprayed with dicamba. RR, RS, SS represent homozygous-, heterozygous-resistant and sensitive kochia plants from a segregating dicamba resistant kochia line 9425. WT represent a field collected dicamba sensitive kochia line. 1-3 represent leaf discs from three individual plants from four kochia genotypes. The top panels are photos of the leaf discs taken before incubation and 36h after being incubated in herbicide solutions under light. Middle panels are corresponding chlorophyll images for the leaf discs in the top panel. Bottom panel are photos of sprayed plants from which the leaf discs were sampled from (4 leaf punches per plant).

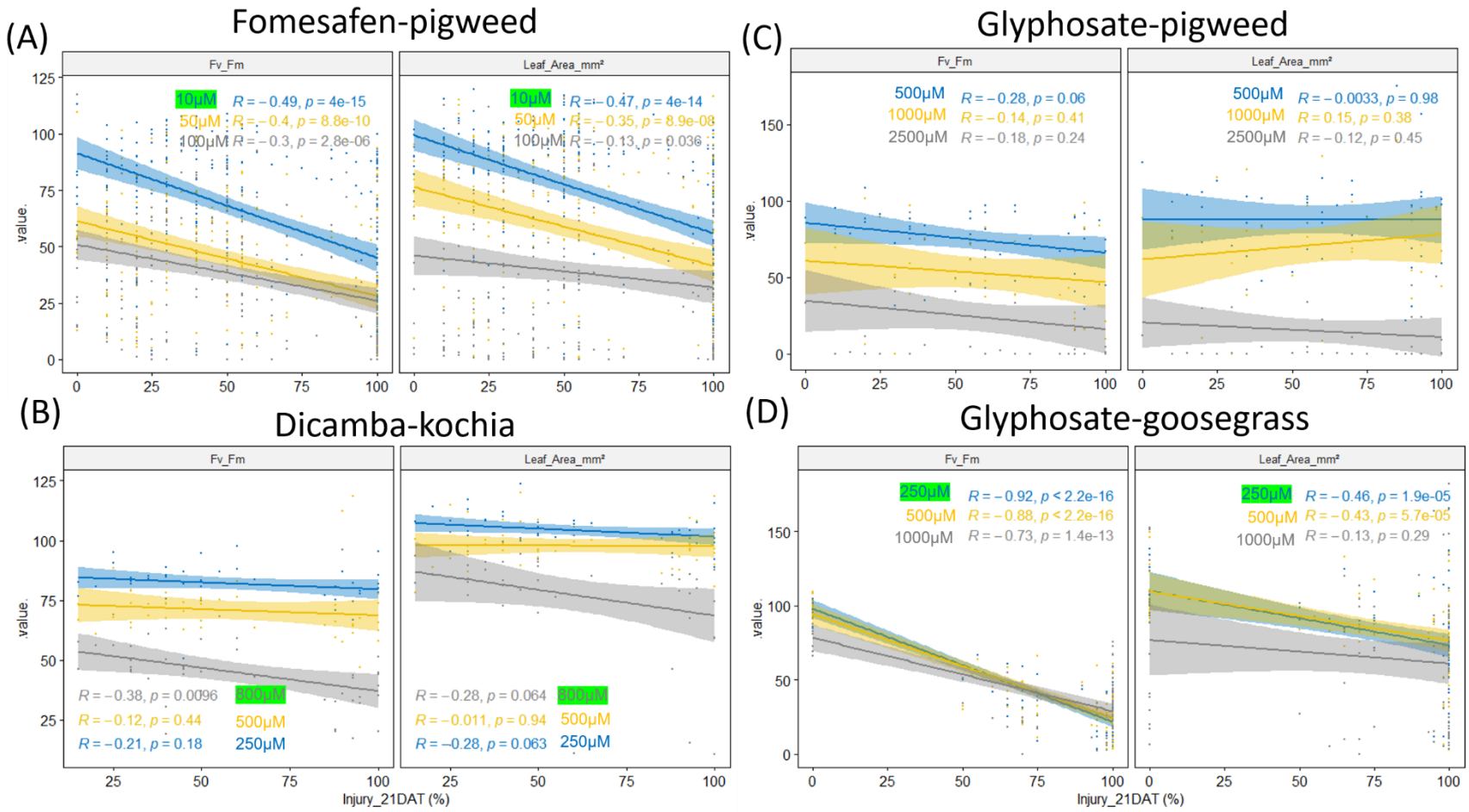

Figure S3. Regression lines showing the correlation between the Fv/Fm values and leaf area (expressed as a percentage of the untreated plants) with spray visual injuries. (A) fomesafen-pigweed, doses: 10, 50, 100μM; (B) dicamba-kochia, doses: 250, 500, 800μM; (C) glyphosate-pigweed; doses: 500, 1000, 2500μM; (D) glyphosate-goosegrass, doses: 250, 500, 1000μM. In each figure, different colors of regression lines indicate doses from low to high: blue, yellow and grey. Discriminative doses for each herbicide are highlighted in green.

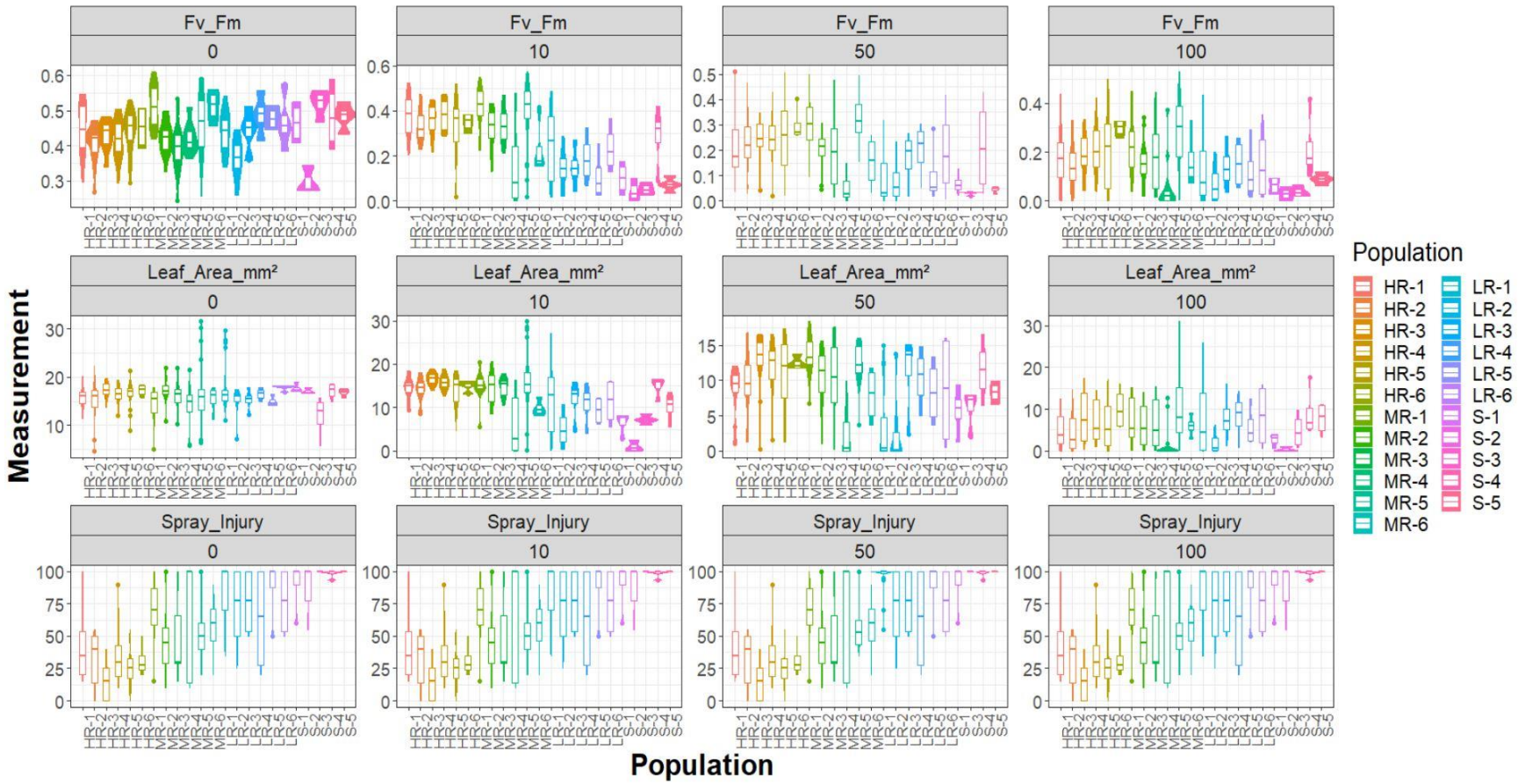

Figure S4. Violin- and box-plots showing Fv/Fm, leaf area and spray injuries of different pigweed populations across different fomesafen doses.

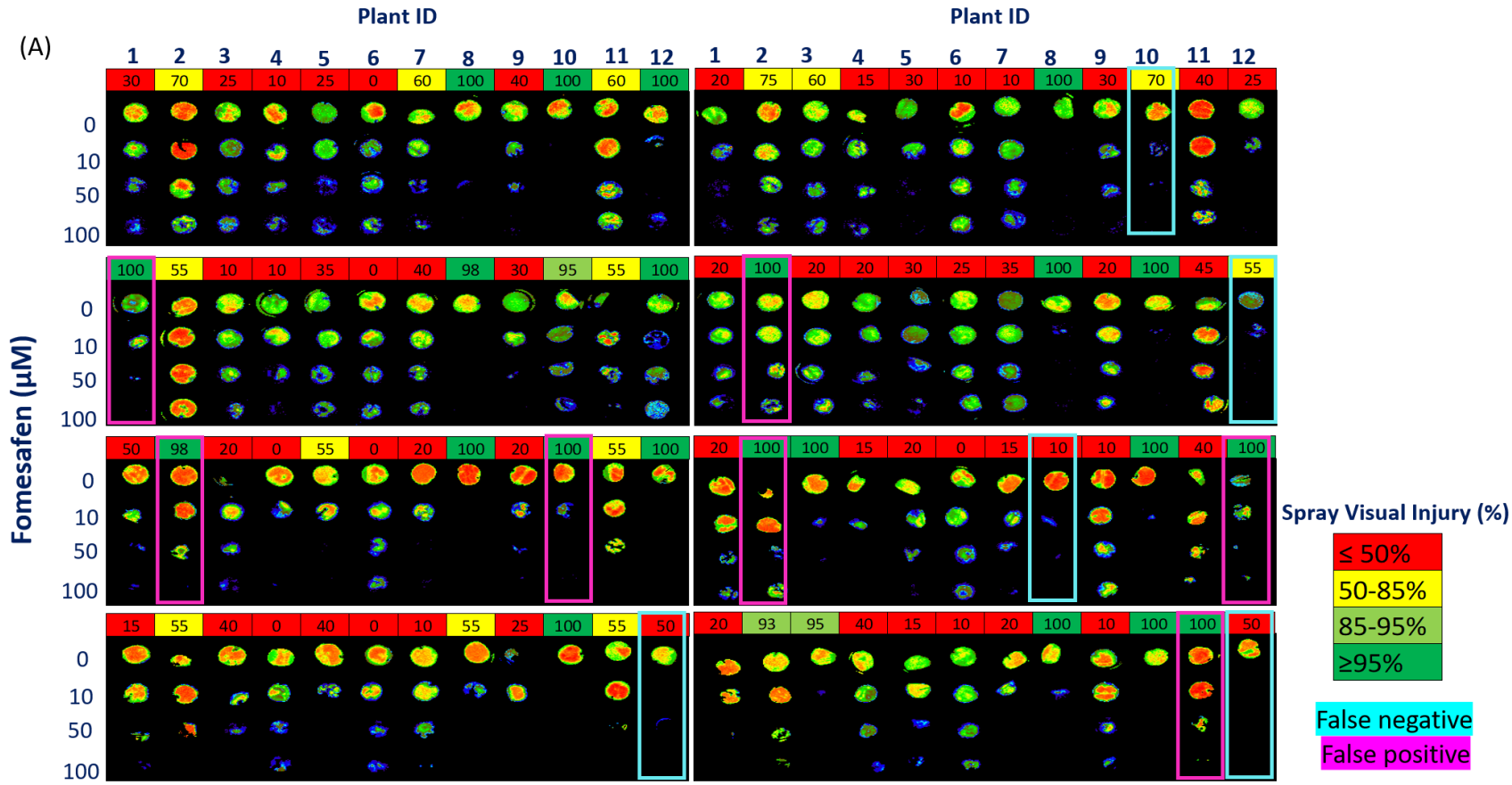

Figure S5. Leaf disc chlorophyll fluorescence images and visual injuries of individual pigweed plants sprayed with fomesafen (Pop 1-12). Plant 1-12 in each panel represent an individual plant from 12 different pigweed populations (8 reps x 12 populations=96 plants in total). For each plant, four leaf discs were challenged with different doses of fomesafen (0, 10, 50, 100 µM) and chlorophyll images were taken 24h after being incubated in herbicide solutions under light. Visual injuries of each plant 14 days after application of 1x rate of fomesafen were indicated on top of each panel and colored coded based on resistance levels. False negative (low Fv/Fm values for resistant plants) and false positive (high Fv/Fm values for susceptible plants) results from the leaf disc assay are highlighted in cyan and magenta rectangular, respectively.

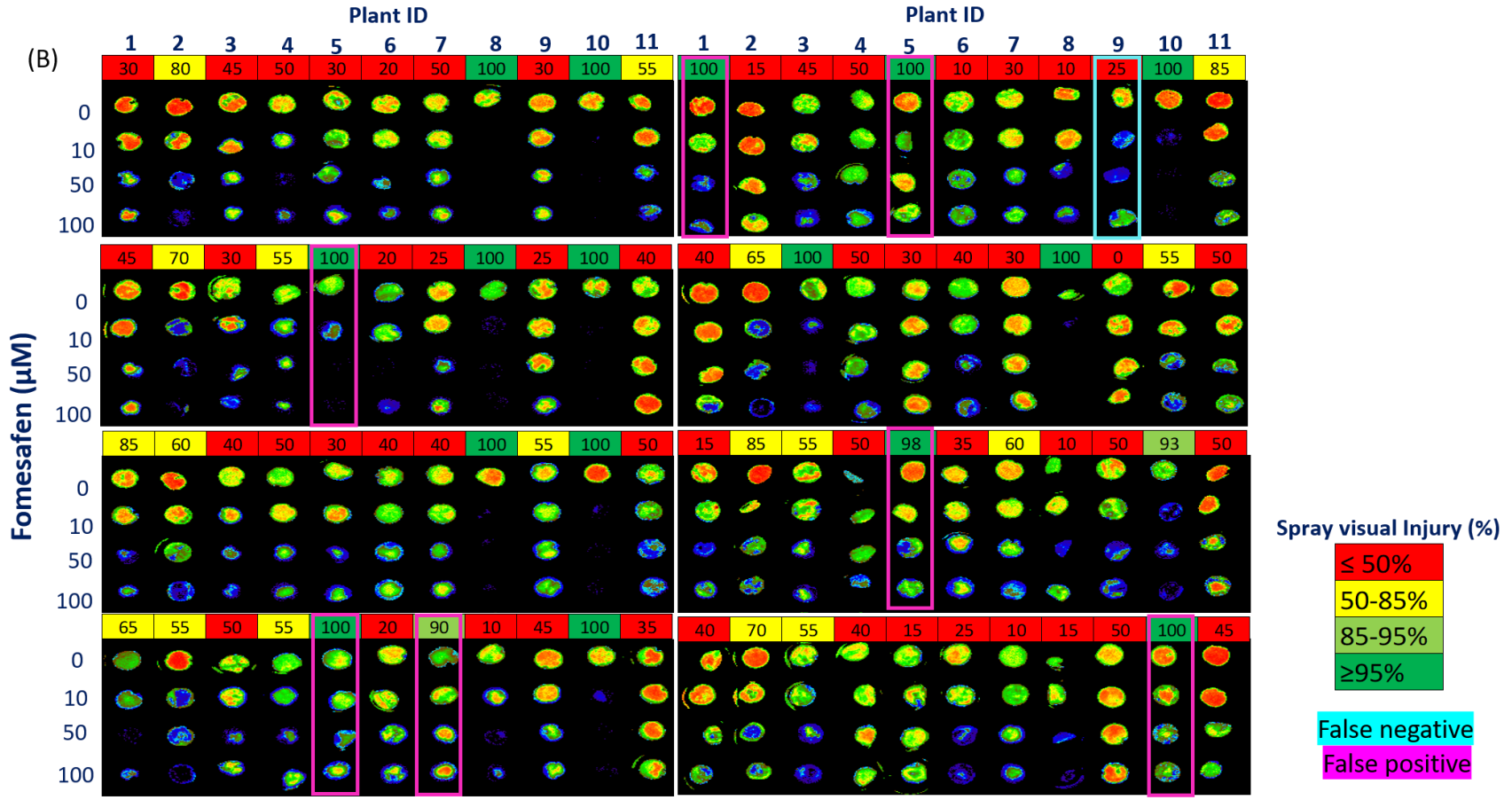

Figure S6. Leaf disc chlorophyll fluorescence images and visual injuries of individual pigweed plants sprayed with fomesafen (Pop 1-11). Plant 1-11 in each panel represent an individual plant from 11 different pigweed populations (8 reps x 11 populations=88 plants in total). For each plant, four leaf discs were challenged with different doses of fomesafen (0, 10, 50, 100 µM) and chlorophyll images were taken 24h after being incubated in herbicide solutions under light. Visual injuries of each plant 14 days after application of 1x rate of fomesafen were indicated on top of each panel and colored coded based on resistance levels. False negative (low Fv/Fm values for resistant plants) and false positive (high Fv/Fm values for susceptible plants) results from the leaf disc assay are highlighted in cyan and magenta rectangular, respectively.

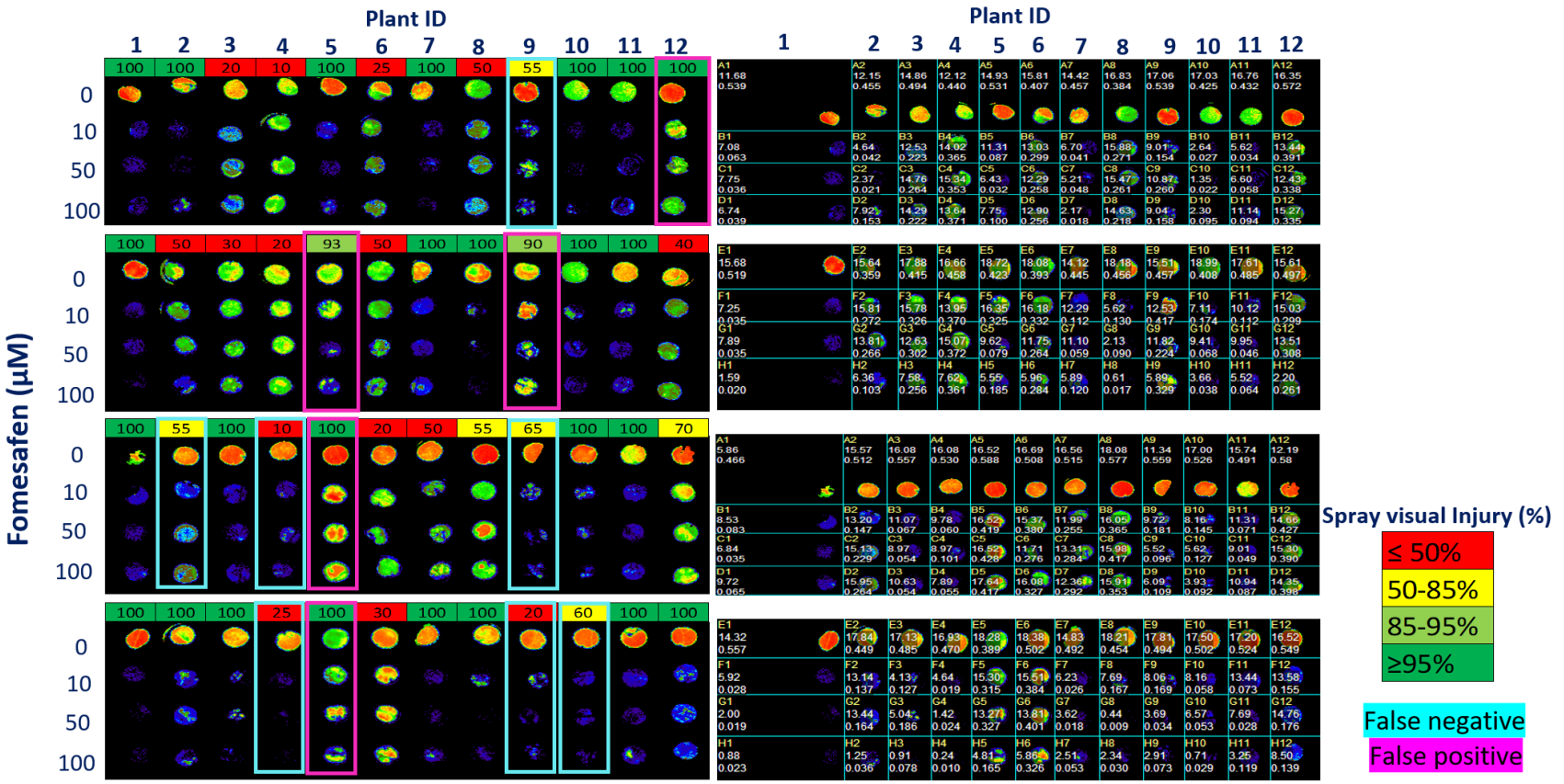

Figure S7. Leaf disc chlorophyll fluorescence images and visual injuries of individual pigweed plants sprayed with fomesafen (Pop 9, 11 & 13-22). Plant 1-12 in each panel represent an individual plant from 12 different pigweed populations (4 reps x 12 populations=48 plants in total). For each plant, four leaf discs were challenged with different doses of fomesafen (0, 10, 50, 100 µM) and chlorophyll images were taken 4h after being incubated in herbicide solutions under light. Visual injuries of each plant 14 days after application of 1x rate of fomesafen were indicated on top of each panel and colored coded based on resistance levels. The panels on the right are same images as the leaf panels but with the quantitative data. False negative (low Fv/Fm values for resistant plants) and false positive (high Fv/Fm values for susceptible plants) results from the leaf disc assay are highlighted in cyan and magenta rectangular, respectively.

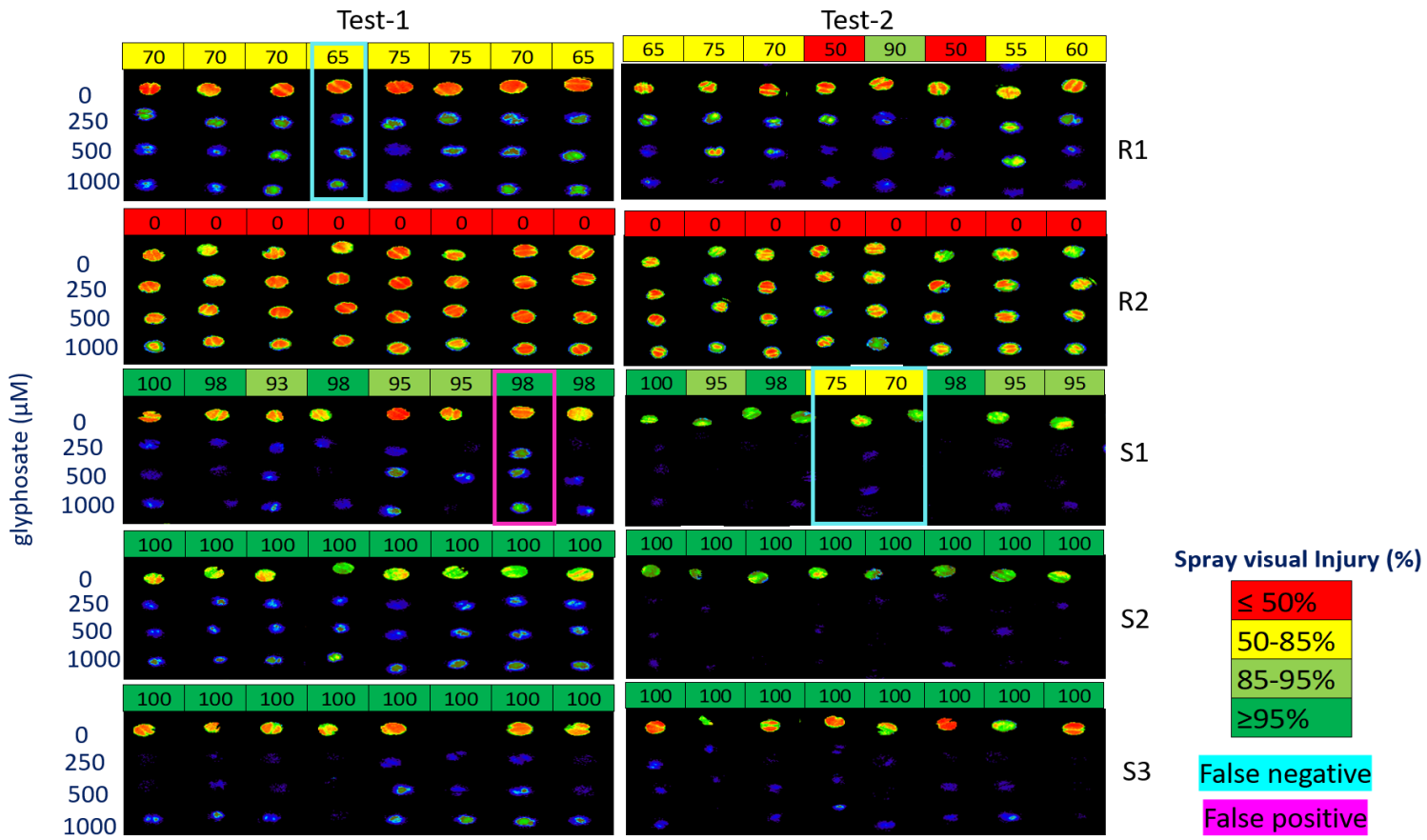

Figure S8. Leaf disc chlorophyll fluorescence images and visual injuries of individual goosegrass plants sprayed with glyphosate. Plant 1-8 in each panel represent an individual plant from 5 different goosegrass populations (8 reps x 2 tests x 5 populations=80 plants in total). For each plant, four leaf discs were challenged with different doses of fomesafen (0, 250, 500, 1000 μM) and chlorophyll images were taken 38h (test-1) and 51h (test-2) after being incubated in herbicide solutions under light. Visual injuries of each plant 21 days after application of 1x rate of glyphosate were indicated on top of each panel and colored coded based on resistance levels. The panels on the right are same images as the leaf panels but with the quantitative data. False negative (low Fv/Fm values for resistant plants) and false positive (high Fv/Fm values for susceptible plants) results from the leaf disc assay are highlighted in cyan and magenta rectangular, respectively.

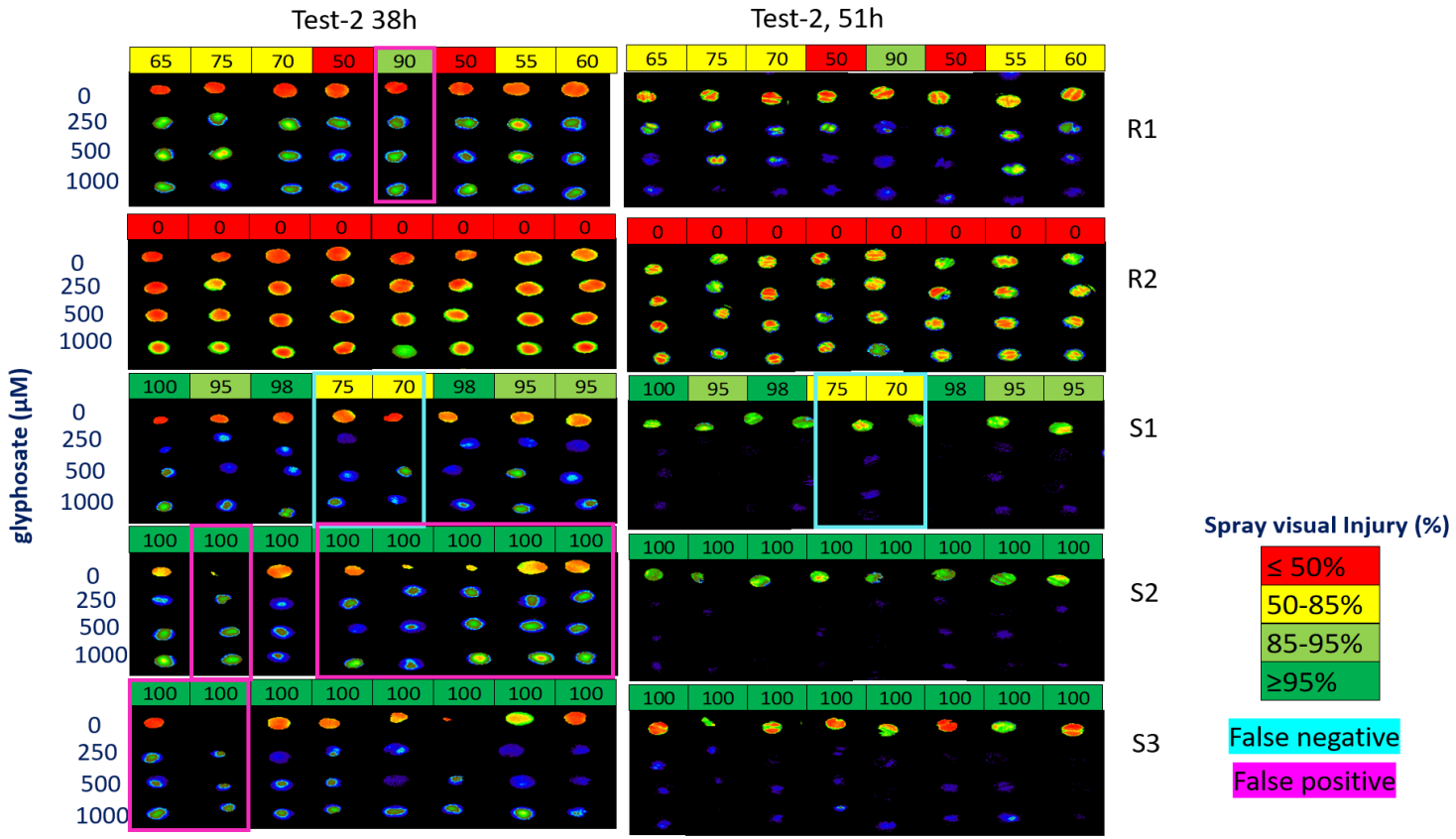

Figure S9. Leaf disc chlorophyll fluorescence images and visual injuries of individual goosegrass plants sprayed with glyphosate at different evaluation time points. Plant 1-8 in each panel represent an individual plant from 5 different goosegrass populations (8 reps x 2 tests x 5 populations=80 plants in total). For each plant, four leaf discs were challenged with different doses of fomesafen (0, 250, 500, 1000 μM) and chlorophyll images were taken 38h (test-1) and 51h (test-2) after being incubated in herbicide solutions under light. Visual injuries of each plant 21 days after application of 1x rate of glyphosate were indicated on top of each panel and colored coded based on resistance levels. The panels on the right are same images as the leaf panels but with the quantitative data. False negative (low Fv/Fm values for resistant plants) and false positive (high Fv/Fm values for susceptible plants) results from the leaf disc assay are highlighted in cyan and magenta rectangular, respectively.