Research Article

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Identification of glyphosate resistance in *Salsola tragus* in north-eastern Oregon

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Abstract

BACKGROUND: Farmers in the low-rainfall region of eastern Oregon rely on repeated applications of non-selective herbicides, predominately glyphosate, to control *Salsola tragus* in no-till fallow systems. Reports of poor glyphosate effectiveness have increased in recent years. Reduced efficacy is often attributed to dust, water stress, or generally poor growing conditions during application. Inadequate control also may be the result of the evolution of glyphosate resistance. Therefore, studies were undertaken to determine if glyphosate-resistant *S. tragus* populations occur in Oregon.

RESULTS: Results from dose–response studies confirmed glyphosate resistance in three of 10 Oregon *Salsola tragus* populations. The ratio I_{50R}/I_{50S} from dose–response curves was, on average, 3.1 for the relative dry biomass per plant and 3.2 for the % of surviving plants per pot in these three populations. Plant mortality at recommended glyphosate doses for the resistant populations was less than 30% 3 weeks after treatment.

CONCLUSIONS: Glyphosate resistance in *S. tragus* highlights the imperative need to diversify weed control strategies to preserve the longevity and sustainability of herbicides in semi-arid cropping systems of the Pacific Northwest.

Keywords: glyphosate-resistant; *Salsola kali*; Russian thistle; tumbleweed

1 INTRODUCTION

Salsola tragus L. [synonym of *Salsola australis* R. Br., *S. iberica* (Sennen & Pau) Botsch. ex Czerep., *S. kali* L., *S. kali* L. subsp. *tragus* (L.) Nyman],[1] is an important weed that causes serious production problems in crops, after harvest, and during the fallow year in the dryland small-grain producing areas of the United States and Canada.[2] In the Pacific Northwest (PNW) of the US, where wheat–summer fallow is the predominant cropping system, *S. tragus* infests nearly two million hectares[3] and costs farmers more than $50 million annually in control measures.[4]

In arid and semi-arid regions, *Salsola tragus* inhabits disturbed areas, such as overgrazed rangeland, abandoned cropland, roadsides, and ditches. These ruderal areas where control measures are more infrequent or do not occur can often be the origin of reintroductions into fields due to its mechanism of seed dispersal. *Salsola tragus* plants are characterized by high seed production, i.e. over 50 000 seeds per plant, and the ability to spread seed widely due to tumbling at plant maturity.[1,5]

The control of *S. tragus* postharvest and in summer fallow is critical to avoid the production of large quantities of seed.[6] The 100 L of soil moisture per plant removed from the soil profile can prohibit crop production in the following year.[2] In the PNW to prevent soil erosion, *S. tragus* control is more commonly accomplished by broadcast applications of nonselective herbicides than by tillage.

In the late 1980s, sulfonylurea-resistant *S. tragus* was reported first in Washington[7] and Kansas[8] and resistance in this species to that group of herbicides also has been reported in Canada and USA.[9,10] Today, glyphosate is the herbicide of choice for growers in the PNW to control *S. tragus* after harvest and in summer fallow.

Long-term reliance on glyphosate has selected other resistant weed species of the inland PNW. Glyphosate-resistant *Lolium multiflorum* and glyphosate-resistant *Kochia scoparia* have been documented in Oregon.[11,12] The decrease in the retail price of glyphosate has encouraged producers to increase the number of applications of this chemical, particularly in no-till chemical fallow systems, thereby further increasing the potential for selection of glyphosate-resistant weed species.

In fall 2015, Oregon farmers reported lack of control of *S. tragus* populations with glyphosate. Therefore, this research was undertaken to determine whether there were glyphosate-resistant *S. tragus* populations in Oregon.

2 MATERIALS AND METHODS

2.1 Collection of Russian thistle populations

Sampling was conducted in February 2016 on fallow fields in Umatilla, Morrow, and Sherman Counties of Oregon (Fig. 1). Plants

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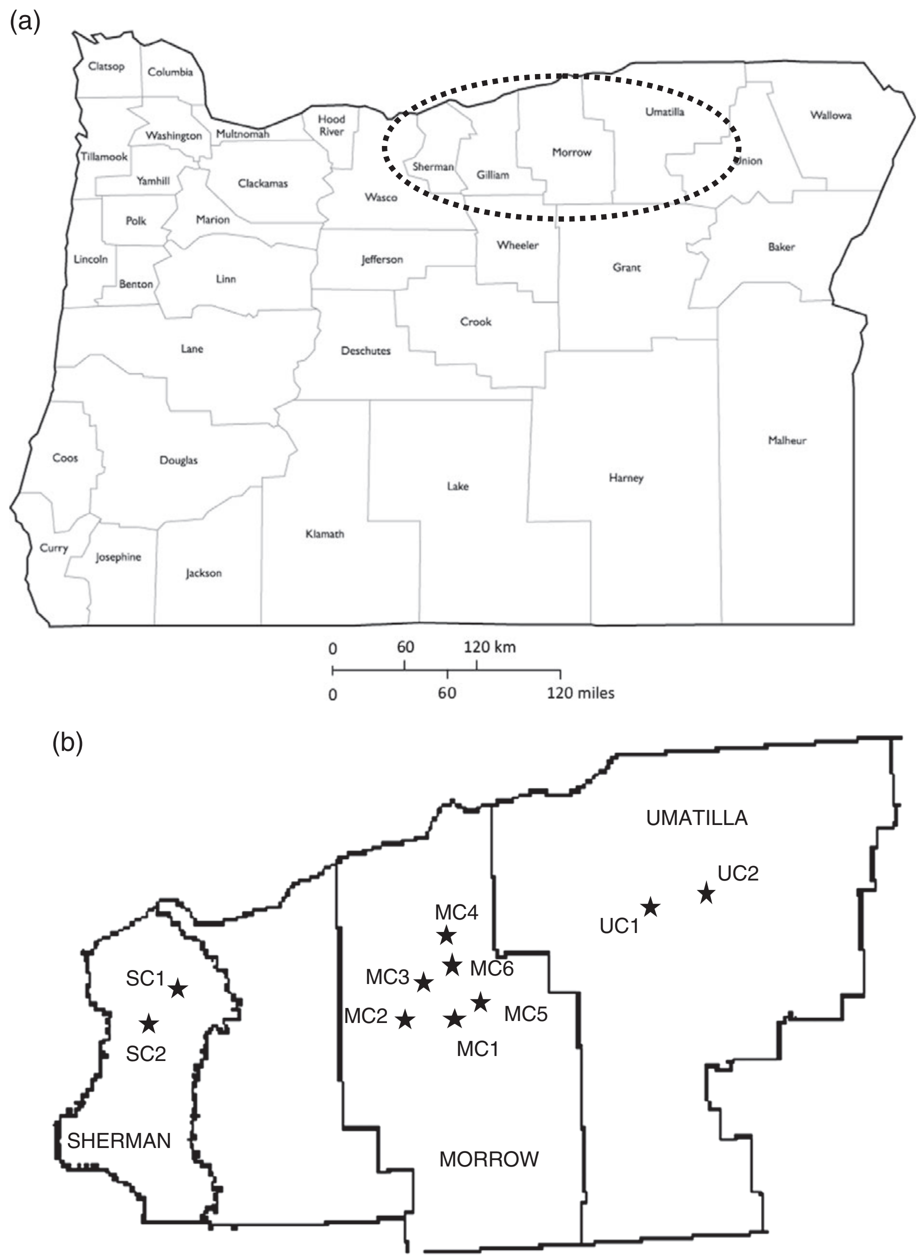

Figure 1. Location of: (a) counties in Oregon state (USA) where samples were taken, and (b) populations collected and tested.

from 10 populations were collected, eight from private fields and two from Oregon State University (OSU) research stations. One of the populations was collected from an organic field that had been in organic production for more than 10 years and was used as the susceptible control population. Only one population was collected per fallow field. Each population consisted of at least 10 randomly collected *S. tragus* plants. Population identifiers are provided in Fig. 1. The seed was threshed and cleaned at the Columbia Basin Agricultural Research Center (CBARC) at Pendleton, OR. Seeds from each population were bulked. Half of the populations were tested at CBARC and the other half were tested at OSU campus (Corvallis, OR) due to limited greenhouse space at CBARC.

2.2 Resistance testing

Before initiating the study, a germination test was conducted on each population. All of the populations had at least 60% germination. Populations UC1, UC2, MC1, MC2, MC6 and SC2 were tested in a greenhouse at CBARC and populations UC1, MC3, MC4, MC5 and SC1 were tested in an OSU campus greenhouse (Corvallis). Greenhouse conditions were similar with temperatures ranging from 13 °C to 26 °C depending on exterior conditions. Natural light was supplemented with artificial light (98 W m^{-2}) from 06.00 hours to 18.00 hours.

The experimental design was a randomized complete block (blocked by population) with treatments replicated six times. Ten seeds were seeded per pot and thinned before treatment to four plants per pot, except for population MC3 which only had one plant per pot due to lack of seed. Pots 15 cm in diameter and 12.5 cm in height (2209 cm^3) were filled with an all-purpose potting soil enriched with controlled release fertilizer 0.13 N, 0.04P, 0.13 K. Plants were treated at doses of 0, 131, 263, 525, 1050, 2100 and 4200 g ae ha^{-1} at CBARC and of 0, 245, 490, 980, 1960 3920 and 7840 g ae ha^{-1} at OSU-campus using the same commercial glyphosate product (Gly Star Original®; Albaugh, LLC., Ankeny, Iowa, USA). Labeled recommended rates for *S. tragus* control range from 840 to 1120 g ae ha^{-1}. Plants were watered as needed.

Herbicide treatments were applied to plants at the five-leaf stage using a compressed air, greenhouse cabinet sprayer with a single 8002E nozzle delivering 96 L ha^{-1} at 242 kPa at CBARC and 140 L ha^{-1} at 242 kPa at OSU-campus. Plants were watered in the morning and treated in the afternoon and were not watered again for 24 h. Plants were watered daily until termination of the study.

Evaluation was conducted 3 weeks after treatment (3 WAT). Live plants per pot were counted, clipped and placed in paper bags. The samples were dried in an oven at 50 °C for at least 48 h and then weighed. The studies were repeated.

2.3 Data analysis

The response of relative dry biomass per plant (calculated as percent of the untreated control plants per population) and percentage of live plants per pot (y) to the herbicide doses (x) were analyzed with the log–logistic function described by Seefeldt *et al.*[13] according to Eqn 1 and the log–logistic function described by Brain and Cousens[14] according to Eqn 2 which adjusted for a hormesis effect:

$$y = C + \frac{D - C}{1 + \left(\frac{x}{I_{50}}\right)^{b}} \quad (1)$$

$$y = C + \frac{D - C + \gamma x}{1 + \left(\frac{x}{I_{50}}\right)^{b}} \quad (2)$$

where C is the lower limit corresponding to a very high dose of herbicide, D is the upper limit corresponding to the untreated control, b is the slope of the curve around the I_{50}, which is the dose that causes an inhibition of 50% with respect to the untreated control, and γ is a parameter that permits a simple test for hormesis [if $\gamma = 0$, Eqn 2 reduces to Eqn 1]. Model parameters were estimated using the base package of R program v. 3.2.1.[15] The level of resistance was determined by calculating an R/S ratio [I_{50} of a resistant (R) biotype divided by the I_{50} of a susceptible (S) biotype].

3 RESULTS

Analysis of variance indicated no effect of experiment per population; therefore, data from the two experiments per population in each greenhouse were combined for analysis of relative dry biomass per plant and percentage of live plants per pot.

3.1 Relative dry biomass per plant after glyphosate treatments

The relative dry biomass per plant showed a log–logistic response to increasing glyphosate doses (Fig. 2, Table 1). Results from each location are shown separately because the herbicide effect on the control population, UC1, was significantly different ($P = 0.01$) (analysis not shown).

Seven populations were controlled with the recommended dose of glyphosate (840 g ae ha^{-1}) for plants smaller than 15 cm, the size of the *S. tragus* seedlings at the time of treatment. In those seven populations, the relative dry biomass per plant was reduced by more than 90% on average (range 75% to 97%). The recommended dose for plants smaller than 30 cm (1120 g ae ha^{-1}) reduced the biomass of these populations more than 95% on average (range

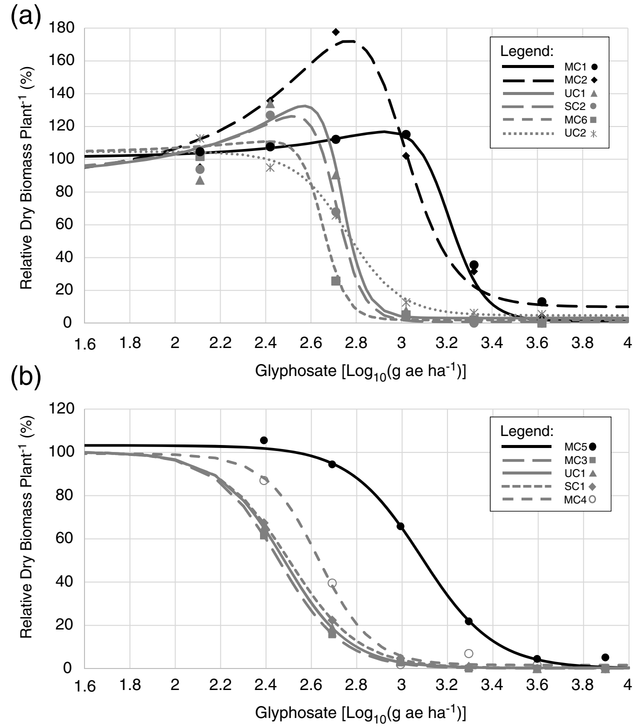

Figure 2. Dose–response curves of relative *S. tragus* dry biomass per plant 3 weeks after treatment. (a) Populations tested at CBARC, and (b) populations tested at OSU-campus. Points indicate mean of the experimental data and lines fitted models (equations and parameters are in the text and Table 1).

89% to 99%). Therefore, these populations were susceptible to glyphosate. However, three populations (MC1, MC2, and MC5) were not controlled by the recommended glyphosate doses. The glyphosate dose of 840 g ae ha^{-1} reduced the relative dry biomass per plant by a maximum of 25% but averaged 0% due to a marked hormetic effect in two of the three populations (Fig. 2). The glyphosate dose of 1120 g ae ha^{-1} reduced the relative dry biomass per plant a maximum of 43% and averaged 20%. Based on the I_{50} values of these three populations, the R/S ratio was between 2.37 and 4.03 (Table 2). The population UC1 (present in both greenhouses), which was confirmed to be susceptible to glyphosate, was used as the susceptible population (S) to calculate R/S ratio.

3.2 Plant mortality after glyphosate treatments

Increasing glyphosate doses decreased the number of live plants per pot (Fig. 3, Table 3). Results from both locations are shown together because the herbicide effect on the control population, UC1, was not significantly different between sites ($P = 0.58$) (analysis not shown). However, glyphosate dose effects differed depending on the population.

The same three populations, MC1, MC2, and MC5, which were not controlled based on the analysis of their relative dry biomass per plant also had significantly lower plant mortality. On average for a dose of 980 g ae ha^{-1}, populations UC1, UC2, SC1, SC2, MC4, MC6, and UC1$_{Cor}$ (population UC1 tested in Corvallis) had 77% mortality (varying between 70% and 93%), while populations MC1, MC2, and MC5 had 18% mortality (varying between 12% and 25%). At double the recommended dose (1960 g ae ha^{-1}), populations UC1, UC2, SC1, SC2, MC6, MC4, and UC1$_{Cor}$ were controlled on

Table 1. Parameters of dose–response models for relative dry biomass per plant of *S. tragus* 3 weeks after treatment with glyphosate

Population	Location	*D*	*C*	I_{50}	*b*	*y*
MC1	CBARC	101 (±8)***	1.2 NS	1769 (±225)***	13.0 (±8)*	0.023 NS
MC2	CBARC	87 (±8)***	9.9 NS	1469 (±228)***	9.8 (±3)**	0.19 (±0.04)***
MC6	CBARC	104 (±4)***	1.9 NS	464 (±19)***	19.2 (±6)**	0.032 NS
UC1	CBARC	90 (±6)***	3.1 NS	619 (±244)*	19.4 (±4)***	0.13 (±0.06)*
UC2	CBARC	105 (±7)***	4.7 NS	578 (±75)***	8.6 (±4) NS	–
SC2	CBARC	91 (±4)***	0.8 NS	582 (±15)***	17.6 (±5)**	0.12 (±0.02)***
MC3	OSU-campus	100 (±6)***	0.1 NS	286 (±25)***	7.1 (±2)***	–
MC4	OSU-campus	99 (±6)***	1.6 NS	429 (±34)***	8.5 (±2)***	–
MC5	OSU-campus	103 (±7)***	0 NS	1215 (±170)***	6.2 (±2)**	–
UC1	OSU-campus	100 (±4)***	0.3 NS	302 (±21)***	7.0 (±1)***	–
SC1	OSU-campus	100 (±5)***	0.2 NS	317 (±27)***	6.5 (±1)***	–

***$P < 0.001$;
**$P < 0.01$;
*$P < 0.05$; not significant (NS), $P > 0.05$.
Eqn 2 was the equation for populations MC1, MC2, MC6, UC1 and SC2, and Eqn 1 was the equation for the other populations.
Value in parenthesis is the standard error of the parameter.

Table 2. Ratio I_{50R}/I_{50S} for relative dry biomass per plant (R/S_{UC1}) and percentage of live plants per pot (R_{lp}/S_{UC1}) of *S. tragus* populations 3 weeks after treatment with glyphosate

Population	Location	R/S_{UC1}	R_{lp}/S_{UC1}
MC1	CBARC	2.86	3.58
MC2	CBARC	2.37	2.80
MC6	CBARC	0.75	1.08
UC1	CBARC	1	1
UC2	CBARC	0.93	0.92
SC2	CBARC	0.94	1.01
MC3	OSU-campus	0.95	–
MC4	OSU-campus	1.42	0.76
MC5	OSU-campus	4.03	3.27
UC1	OSU-campus	1	1
SC1	OSU-campus	1.05	1.11

S_{UC1} is the I_{50} of the susceptible population UC1 that was tested in both locations.
R is the I_{50} for the relative dry biomass per plant of the populations.
R_{lp} is the I_{50} for the percentage of live plants per pot of the populations.

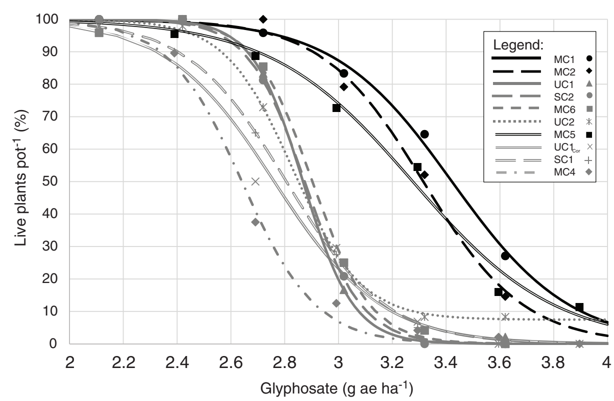

Figure 3. Dose–response curves of percentage of *S. tragus* live plants per pot 3 weeks after treatment. Points indicate mean of the experimental data and lines fitted models (equation and parameters are in the text and Table 3).

average 96% (range 90% to 99%), while control of populations MC1, MC2, and MC5 averaged 45% control (range 36% to 52%). The *R/S* ratios for populations MC1, MC2, and MC5 were between 2.80 and 3.58 (Table 2). These results and those shown previously for the relative dry biomass per plant confirm glyphosate resistance in these three *S. tragus* populations.

4 DISCUSSION

The identification of glyphosate resistance in *S. tragus* is a serious threat to the sustainability of the wheat-summer fallow cropping systems of the Inland Pacific Northwest due to the high frequency of this weed species and the extensive reliance on the use of glyphosate for its control. *Salsola tragus* plants have a high potential to move with the wind direction due to their tumbling nature and can spread seeds over long distances,[5] which may allow the resistance to spread very quickly.

The greater glyphosate effectiveness found for the relative dry biomass per plant in one of the greenhouses could be due to multiple factors such as, average temperature, light intensity, relative air humidity, etc. Some of these variables have been reported to affect herbicide efficacy (see, for example, Kleiman *et al.*,[16] Adkins *et al.*,[17] Stopps *et al.*[18]). However, the fact that the greenhouse with lower herbicide efficacy produced a hormetic effect in most of the tested populations at sub-lethal glyphosate doses might have impacted the differences in the biomass response. Even though the hormesis response to glyphosate has been reported previously,[19–21] the mechanisms involved are still unclear,[22] and we could not identify differences in a variable which might have triggered the hormetic effect in just one of the greenhouses. Nonetheless, the differences in the relative dry biomass per plant in the two greenhouses did not affect *S. tragus* plant mortality or the important findings of this study.

The three populations identified as glyphosate resistant had a relatively low level of resistance (ratio $R/S < 10$). However, the average ratio R_{I50}/S_{I50} for the relative dry biomass per plant (3.1) and percentage of surviving plants per pot (3.2) increased to 6.4 and 4.5, respectively, if calculated at the I_{90} level instead of at the I_{50}. In addition, those ratios are not based on a single *S. tragus*

Table 3. Parameters I_{50} and b (slope of the curve around the I_{50}) of dose–response models for percentage of *S. tragus* live plants per pot 3 weeks after treatment with glyphosate

Population	Location	I_{50}	b
MC1	CBARC	2641 (±165)	4.6 (±0.6)
MC2	CBARC	2066 (±126)	5.4 (±0.7)
MC6	CBARC	800 (±41)	9.4 (±1.4)
UC1	CBARC	738 (±26)	10.6 (±1.1)
UC2	CBARC	677 (±46)	7.9 (±1.4)
SC2	CBARC	749 (±29)	9.5 (±1.0)
MC4	OSU-campus	438 (±21)	7.3 (±1.1)
MC5	OSU-campus	1889 (±173)	3.8 (±0.5)
UC1	OSU-campus	577 (±46)	5.0 (±0.8)
SC1	OSU-campus	640 (±38)	5.5 (±0.7)

Both parameters were significant (P-value < 0.001) for all the populations.
Eqn 1 was the fitted equation to the experimental data for all the populations.
Parameter D was 100% for all populations (P-value <0.001) and parameter C was not significant (value = 0) except for population UC2 [value = 4.7 (P < 0.05)].
Value in parenthesis is the standard error of the parameter.

biotype that survived a glyphosate application but rather from 10 to 50 *S. tragus* plants per population collected randomly. More directed sampling of plants that survived glyphosate treatment might increase those ratios significantly.

In the USA, evolved resistance to glyphosate in *Kochia scoparia*, a species from the same family as *S. tragus* and with a similar mechanism of seed dispersal, was first reported in Kansas in 2007 and subsequently has been found in many other states.[23–25] A similar trend may occur with *S. tragus* unless there is an immediate transition to a more diversified approach for control of this troublesome weed species.

5 CONCLUSIONS

The results of these experiments confirmed glyphosate resistance in three *S. tragus* populations in Oregon. These findings should be considered for management recommendations to control this species, extend the life of glyphosate, prevent the spread of the resistance, and ultimately the sustainability of the affected cropping systems, such as those in the driest region of the Inland Pacific Northwest.

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