TITLE: Evaluation of occurrence of glyphosate resistant Russian thistle (*Salsola tragus*) in Northeastern Oregon

RESEARCH LEADER: Judit Barroso – Weed Scientist
Assistant Professor, Columbia Basin Agricultural Research Center (CBARC)
48037 Tubbs Ranch Road, Oregon State University, Pendleton, OR, 97801

COORDINATORS: Larry Lutcher - Dryland Cereal Production Specialist
Full Professor, Morrow County Extension Office
54173 Hwy 74 P.O. Box 397, Heppner, OR 97836

SUMMARY:
Farmers in the low-rainfall region of eastern Oregon rely on repeated applications of non-selective herbicides, predominately glyphosate, to control Russian thistle 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 Russian thistle populations occur in Oregon. The effect of a glyphosate application post-harvest on this species was also evaluated. Results from dose-response studies confirmed glyphosate resistance in several Russian thistle populations of northeastern Oregon. The ratio *I₅₀R/I₅₀S* from dose-response curves was on average 3.2 for the percent of surviving plants per pot in the greenhouse and 3.8 for the percent of surviving plants per plot in the field. The glyphosate resistance did not cause a fitness cost in seed production and/or seed germination, which could indicate a rapid spread of the resistance if Russian thistle control strategies are not diversified promptly.

OBJECTIVES:
1) Determine the occurrence of glyphosate-resistant Russian thistle populations in Northeastern Oregon.
2) Evaluate the effect of post-harvest glyphosate application on Russian thistle plant mortality, seed production, and seed viability considering two application times.

PROCEDURES:
1) *Determine the occurrence of glyphosate-resistant Russian thistle populations in Northeastern Oregon.*
In February 2016, on fallow fields of Umatilla, Morrow, and Sherman Counties, Larry Lutcher, my technician Jennifer Gourlie and I collected ten populations of Russian thistle in fallow fields (Figure 1 and 2). Eight populations were collected 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 ten years and was used as the susceptible control population. Each population consisted of at least ten Russian thistle plants randomly gathered and labelled for later processing (population identifier is provided in Figure 2). The seed was threshed and cleaned at the Columbia Basin Agricultural Research Center (CBARC). Seeds from each population were bulked.

Greenhouse study. 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 temperatures ranged from 13°C to 26°C depending on exterior conditions. Natural light was supplemented with artificial light (98 W m⁻²) from 6 am to 6 pm.

![Image](image1.jpg)

Figure 1: Collecting Russian thistle populations in fallow fields of Morrow County.

![Image](image2.jpg)

Figure 2: Location in Sherman, Morrow, and Umatilla Counties of the collected populations.
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 cm³) were filled with an all-purpose potting soil enriched with controlled release fertilizer 0.13N - 0.04P - 0.13K. 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®). The doses corresponded to 0X, 0.125X, 0.25X, 0.5X, 1X, 2X, and 4X at CBARC and 0X, 0.25X, 0.5X, 1X, 2X, 4X, and 8X at Corvallis. Labeled recommended rates for Russian thistle control range from 840 to 1120 g ae ha⁻¹ (24 – 32 fl oz A⁻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 242kPa 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 hrs. 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 experiment was repeated.

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 dose-response curves.

Field study. In spring 2017, we established an experiment at Moro and Pendleton research stations in fallow fields to confirm the observations in the greenhouse. We selected two susceptible and two resistant Russian thistle populations from the populations analyzed in the greenhouse. Russian thistle seeds were germinated in the greenhouse and 20 of them per population and treatment were moved to the field at two leaf stage in mid-May. The experimental design was a split-plot randomized complete block with three repetitions. Plots were 10 ft by 55 ft and sub-plots 10ft by 10ft. Russian thistle seedlings were transplanted into the center of each sub-plot in a sub-area of 4 ft by 3 ft (Figure 3). Russian thistle populations were sprayed with glyphosate at 0, 30, 60, and 120 oz/ac (corresponding with 0X, 1X, 2X, and 4X) at 6-7 leaf stage (about 3 inches tall). Three weeks after treatment, survivor plants were counted.

The response of percentage of live plants per plot to herbicide doses was analyzed with dose-response curves.
2) **Evaluate the effect of post-harvest glyphosate application on Russian thistle plant mortality, seed production, and seed viability considering two application times.**

In spring 2016, a field experiment was established in spring wheat at Moro and Pendleton research stations with four of the Russian thistle populations collected in February 2016. The experimental design was a split-split plot randomized complete block with three repetitions (Figure 4), where the main factor (plots) was the application time (2 weeks after harvest or 4 weeks after harvest), the second factor (sub-plots) was the glyphosate rate (0X, 1X, 2X, and 4X) and the third factor (sub-sub-plots) was the Russian thistle population. Sub-sub-plot size was 10 ft by 10 ft, sub-plot size was 10 ft by 55 ft, and plot size was 55 ft by 70 ft. In each sub-sub plot, in March 2016, right before the crop seeding, Russian thistle seeds were sprinkled and incorporated in Pendleton and only sprinkled in Moro. Treatments were applied in 15 gal/ac of water using a CO₂ pressurized sprayer with a 9 ft wide hand-held boom equipped with flat fan nozzles (Teejet XR 8002). Seed production was evaluated 60 DAT by cutting aerial plant biomass from about five live Russian thistle plants per plot. The seed viability was tested in a growth chamber with Petri dishes of 9 cm in diameter with two layers of filter paper, moistened with 10 mL of water, using 25 seeds per dish and 4 dishes per population and treatment. These tests were conducted in a growth chamber under controlled conditions at 22 °C in darkness. Tests were repeated to study the need of a vernalization period.

The response of Russian thistle plant mortality, seed production, and seed viability to post-harvest glyphosate rates and application times were analyzed with ANOVA.
SIGNIFICANT ACCOMPLISHMENTS TO DATE:
1) **Determine the occurrence of glyphosate-resistant Russian thistle populations in Northeastern Oregon.**

Analysis of variance indicated an effect of experiment (greenhouse location) for the relative dry biomass per plant but no effect for the percentage of live plants per pot; therefore, data from the two greenhouses were analyzed independently for the relative dry biomass per plant but they were combined for analysis of percentage of live plants per pot.

The analysis showed that with the recommended glyphosate dose, seven populations were controlled but not three of them (MC1, MC2, and MC5) for the variables studied (Figure 5 and 6). Based on the $I_{50}$ values (which is the dose that causes an inhibition of 50% with respect to the untreated control) of these three populations, the R/S ratio ($I_{50}$ of a resistant (R) biotype divided by the $I_{50}$ of a susceptible (S) biotype) was between 2.4 and 4.0 for the relative dry biomass per plant and between 2.8 and 3.6 for the percentage of live plants per pot. These results confirm glyphosate resistance in these three Russian thistle populations. Pictures of a susceptible versus resistance population can be found in Figure 7.
Figure 5. Dose-response curves of relative Russian thistle 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. Results from each location are shown separately because the herbicide effect in the control population, UC1 – tested in both sites, was significantly different.
Figure 6. Dose-response curves of percentage of Russian thistle live plants per pot 3 weeks after treatment. Points indicate mean of the experimental data and lines fitted models. Results from both locations are shown together because the herbicide effect in the population control (UC1 – present in both greenhouses) was not significantly different.

Figure 7. Photos of the seven treatments 0X (white label), 0.125X (yellow label), 0.25X (blue label), 0.5X (green label), 1X (pink label), 2X (orange label), and 4X (red label) sprayed on a) a susceptible population from Umatilla County and b) a resistant population from Morrow County.
The experiment conducted in Pendleton and Moro in 2017 confirmed the glyphosate resistance in Russian thistle populations under field conditions. The ratio $I_{50R}/I_{50S}$ from dose-response curves for the percent of surviving plants per plot was on average 4.9 in Pendleton and 2.7 in Moro. Plant mortality at the recommended glyphosate dose (1X) for the resistant populations was less than 65% and 85.2% on average three weeks after treatment (Figure 8). In Moro, the same populations that were established in Pendleton, were more resistant (had higher percentage of survivors), which indicates that environmental conditions of the location play an important role on herbicide effect.

![Relative Live Plants After a Spring Application in Pendleton](image1.png)

![Relative Live Plants After a Spring Application in Moro](image2.png)

Figure 8. Percentage of live plants per plot three weeks after herbicide treatment at the Pendleton experiment (a) and at the Moro experiment (b). Green bars indicate populations that
were susceptible in the greenhouse and red bars indicate population that were resistant in the greenhouse. Numbers on the bars indicate the value of the mean and bar whiskers indicate the standard error of the mean.

2) **Evaluate the effect of post-harvest glyphosate application on Russian thistle plant mortality, seed production, and seed viability considering two application times.**

The results from the ANOVA analysis showed that there was not a significant effect in plant mortality, seed production, and seed viability with the application times (glyphosate applied two or four weeks after harvest) in any of the studied locations (Pendleton and Moro).

**Plant mortality.** At label rate, glyphosate did not control either resistant nor susceptible populations (percentage of live plants was always greater than 50% with 1X). Contrarily to what we observed after a spring treatment, glyphosate was more effective in Moro than in Pendleton (Figure 9). The main difference between both experiments that could have affected
Figure 9. Percentage of live plants 45 days after glyphosate treatments that were applied two (light green and red bars) and four (dark green and dark red) weeks after harvest at the Pendleton experiment (a) and at the Moro experiment (b). Green bars indicate the average of susceptible populations and red bars indicate the average of resistant populations.

Herbicide efficacy was that the crop was cut shorter in Moro than in Pendleton. However, further investigations are needed to confirm this observation. With the label rate (recommended rate), it was not possible to distinguish between susceptible and resistant Russian thistle populations after a postharvest application. However, it was possible when the rate was double (2X) in Pendleton and quadruple (4X) in Moro.

Seed production. Seed production showed an effect with location (Pendleton versus Moro) and population (resistant versus susceptible) (P<0.005) (Table 1). Seed production was higher in Pendleton than in Moro. This could possibly be because the Russian thistle plants in Moro were more damaged at harvest than in Pendleton, however, further investigation is needed with this respect. Resistant populations showed higher seed production on average than susceptible populations (Table 1) and their seed production was not affected by the glyphosate rate. Glyphosate only affected seed production in the susceptible populations at the Pendleton experiment when the plants were treated with 4X (P<0.005). This high rate did not produce survivor plants in Moro.

Considering only the control plots (0X), without the presence of glyphosate, seed production was only marginally higher (α > 0.1) for the resistant population (117.0 on average) than for the susceptible one (113.9 on average) and similar for both locations.

Table 1. Mean of the Russian thistle seed production (seeds per gram of aerial plant biomass) and seed germination (%) by location and type of population (S = Susceptible and R = Resistant). Different capital letters indicate significant differences (α < 0.05) for the main effect of location or population and different minor cases letters indicate significant differences for the interaction between those two factors.

<table>
<thead>
<tr>
<th>Seed production (Seed per g of plant biomass)</th>
<th>Seed germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location Population</td>
<td>Pendleton</td>
</tr>
<tr>
<td>S</td>
<td>110a</td>
</tr>
<tr>
<td>R</td>
<td>145b</td>
</tr>
<tr>
<td>Mean</td>
<td>128A</td>
</tr>
</tbody>
</table>

* P-value was 0.06. It was not significant for α < 0.05 but it was for α < 0.1.

Seed viability/germination. Similarly to seed production, seed viability was significant (α < 0.05) with the location and marginally significant (α < 0.1) with the Russian thistle population (susceptible or resistant), but it was not significant with the glyphosate rate (Table 1). Seeds produced in Moro had on average 78% of germination versus a 67% of germination in Pendleton. Seeds did not need a vernalization period to germinate; the germination percentage was similar with fresh seeds vs. seeds that were kept in the refrigerator for two months. The
glyphosate-resistant populations produced seeds with slightly higher germination percentage (77%) vs. the susceptible populations (68%) (Table 1).

Considering only the control plots (0X), without the presence of glyphosate, seed germination in relation to location and population type showed the same response than when all rates were considered in the analysis. Seed germination was significantly higher in Moro (84% on average) than in Pendleton (68% on average) and was only marginally higher (α > 0.1) for the resistant populations (80.5% on average) than for the susceptible ones (71.5% on average).

BENEFITS & IMPACTS

The results of experiments in objective 1 confirmed glyphosate resistance in some Russian thistle populations in Oregon.

The results of this objective highlight the imperative need to diversify Russian thistle control strategies to 1) preserve the longevity and sustainability of glyphosate and 2) prevent the spread of resistant populations, in semi-arid cropping systems of the Pacific Northwest.

The results of experiments in objective 2 indicated an absence of fitness cost in seed production and seed germination in the resistant populations compared with susceptible populations. In fact, the resistant biotypes showed higher seed production and seed germination than the susceptible biotypes.

The results of the second objective indicate a potential quick spread of the glyphosate resistance in Russian thistle due to it does not seem to produce a fitness cost to the plant.

The benefits of this project are that growers will be aware of this problem and can react to it. First, they will stop wasting money on glyphosate to control Russian thistle infestations, particularly on post-harvest applications and second, they will improve their Russian thistle management by using control alternatives to glyphosate.

A scientific article titled "Identification of glyphosate resistance in Salsola tragus in Northeastern Oregon" has been published in Pest Management Science (DOI: 10.1002/ps.4525) (2017) to show the results of this project.

Several extension publications have been published to spread the findings:

I have also been invited to give several seminars on the results of the project:


Several articles have been published in local newspapers to mention some of the work conducted in this project:

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:
An additional $9,173 dollars was awarded by the Oregon Wheat Commission (OWC) to support the field experiments in objective 1 the second year of this project. During the second year of this project and because of the findings from the first year, OWC also awarded $5,000 to initiate research on alternatives herbicide to glyphosate to control Russian thistle.

FUTURE FUNDING POSSIBILITIES:

Pending
- Prevention of Russian thistle (Salsola tragus) dispersion and field re-infestation. Submitted to ARF for the project cycle 2018 – 2020.

In preparation
- Russian Thistle Management in Wheat Cropping Systems. To submit to OWC for the fiscal year 2019.

The next step will be to pursue a NIFA project in cooperation with other states (WA, MO, and ID) to improve Russian thistle management at a regional scale.