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Short title: *Salsola tragus* ecology

Russian thistle (*Salsola tragus*) ecology in wheat cropping systems of the Pacific Northwest

Fernando H. Oreja¹, Nicholas G. Genna², Jennifer A. Gourlie³, and Judit Barroso⁴

¹Postdoctoral Research Associate (ORCID 0000-0002-2553-3802), Oregon State University, Columbia Basin Agricultural Research Center, Adams, OR, USA.

²Postdoctoral Scholar (ORCID 0000-0001-8924-9870), Oregon State University, Columbia Basin Agricultural Research Center, Adams, OR, USA.

³Senior Faculty Research Assistant, Oregon State University, Columbia Basin Agricultural Research Center, Adams, OR, USA.

⁴Associate Professor (ORCID 0000-0002-4453-275X), Oregon State University, Columbia Basin Agricultural Research Center, Adams, OR, USA.

Author for correspondence: Fernando Oreja, Postdoctoral Research Associate, Oregon State University, Columbia Basin Agricultural Research Center, Adams, 48037 Tubbs Ranch Rd., OR, 97810. (Email: foreja@clemson.edu)

Abstract

Russian thistle (*Salsola tragus* L.) is a significant summer annual weed in the semi-arid Pacific Northwest (PNW), causing yield losses of up to 50%. Understanding the biology and ecology of *S. tragus* is vital for developing effective integrated weed management (IWM) strategies. This study focused on 1) *S. tragus* emergence and seedbank persistence in two cropping systems: fallow–winter wheat (*Triticum aestivum* L.) and spring wheat–fallow–winter wheat rotations, and 2) *S. tragus* plant biomass and viable seed production in fallow and spring wheat fields. A four-year experiment (2020-2023) was conducted at the Columbia Basin Agriculture Research Center (CBARC) in Adams, OR, using a randomized block design with four replications. *Salsola tragus* seeds were sprinkled only at the beginning of the experiment, and seedling numbers were recorded throughout. Most seedlings emerged in the first year, with the highest rates in spring wheat (72%) and fallow (32%), followed by significantly lower rates (0.25-5%) in subsequent years. Seedling emergence began in late March and early April for the first two years but was delayed to May for the third year. Plant biomass and viable seed production were greater in fallow than in spring wheat, with early-season plants having more biomass than later-emerging plants. Plants emerged between early and mid-May produced the most viable seeds. Viable seed production was very low until it peaked in mid-September. Findings indicated that most *S. tragus* seedlings emerged in the first year after dispersal coinciding with spring precipitation and lasting approximately two months. Additionally, most *S. tragus* plants produce viable seeds in September, and seeds persist in the soil for more than two years. These results demonstrate the need for growers to control *S. tragus* emergence to prevent reinfestations and ultimately the need to control *S. tragus* plants before September to prevent the species from producing viable seed.

Keywords: Germination, integrated weed management, seedbank, seedling emergence, wheat, weed ecology.

Introduction

Russian thistle (*Salsola tragus* L.) is a summer annual weed native to arid regions of southeastern Europe and central Asia. It was introduced in South Dakota in 1837 and has since become widely distributed throughout the arid and semiarid regions of the Pacific Northwest (PNW) (Beckie and Francis 2009). In spring wheat, *S. tragus* can drive yield losses of up to 50% in severe infestations (Young 1988). Furthermore, *S. tragus* can regrow after harvest in August and September, leading to future yield losses by using up to 100 L of soil water and producing an average of 46,000 seeds per plant (Schillinger and Young 2000).

Growers in the PNW have overwhelmingly adopted minimal or no-till systems to reduce soil erosion and increase soil water content during fall and winter precipitation periods (Williams et al. 2014). Growers rely on pre- and post-emergence herbicides as the primary method to manage *S. tragus*, such as 2,4-D, bromoxynil, carfentrazone, dicamba, flumioxazin, glufosinate, metribuzin, paraquat, saflufenacil, and sulfentrazone (Lutcher 2015; Kumar et al. 2017; Lyon et al. 2021), but particularly on glyphosate to control *S. tragus* in fallow and/or post-harvest. This reliance on herbicides has selected for herbicide-resistant *S. tragus* biotypes that are resistant to acetolactate synthase (ALS) inhibitors (Heap 2023) and glyphosate (Barroso et al. 2018; Kumar et al. 2017). The escalating prevalence of herbicide-resistant *S. tragus* is diminishing herbicide efficacy in the region and intensifying weed pressure. Consequently, growers are considering a return to traditional tillage as an option to manage this species.

The concept of integrated weed management (IWM) is a holistic strategy that combines various weed control techniques. When applied systematically, this integrated approach achieves more effective weed control than any single method alone (Swanton and Weise 1991). Understanding the biology and ecology of problematic weeds in a region, such as *S. tragus*, is crucial to implementing a successful IWM program (Harker and O'Donovan 2013). For instance, understanding population dynamics, emergence patterns, competitive ability, fecundity, dispersal mechanisms, and seedbank persistence will ensure that management plans focus on the most sensitive life stages of target weeds to achieve the greatest long-term efficacy.

The ecology of a weed growing with or without crop (or depending on the crop type) can differ, not only because of the direct competitive interactions for light, nutrients, and water (Patterson 1995), but also the indirect non-competitive interactions related to changes in

temperature, humidity, or light (Roach and Wulf 1987). Therefore, the environment in which *S. tragus* seeds are found may impact germination, plant development, seed production, and seed viability. This study aimed to characterize *S. tragus* emergence, plant biomass, viable seed production, and seed longevity in the two most common crop rotations (winter wheat (*Triticum aestivum* L.) – fallow and winter wheat – spring wheat/spring barley – fallow rotations) of the semi-arid PNW, where *S. tragus* is most problematic.

Materials and Methods

Experiment description

Two sites were chosen in 2020 to establish a no-till farming experiment at the Columbia Basin Agriculture Research Center (CBARC) (45.7196°N, 118.6225°W) in Adams, Oregon, with two main goals: 1) studying *S. tragus* seedling emergence and seed longevity, and 2) studying plant biomass and timing of viable seed production, both in two cropping systems. One site (45.7198°N, 118.6261°W) followed a fallow-winter wheat rotation (hereafter referred to as F/WW) and the other site (45.7261°N, 118.6261°W) followed a spring wheat-fallow-winter wheat rotation (SW/F/WW) (Table 1).

F/WW and SW/F/WW each had two subsites. Subsites F/WW1 and SW/F/WW1 were initiated in 2020 and subsites F/WW2 and SW/F/WW2 were initiated in 2021. Subsites F/WW1 and F/WW2 began in fallow, while subsites SW/F/WW1 and SW/F/WW2 began in spring wheat. Rotation phases are documented in Table 1 for each year of this study. The experimental design was a randomized complete block design with four replications and ten plots (3 m x 4.5 m) per replication. Nine of the ten plots were sprinkled with 400 *S. tragus* seeds in two, one m² areas in the center of each plot in early March of 2020 (in F/WW1 and SW/F/WW1) and of 2021 (in F/WW2 and SW/F/WW2). The remaining plot per replication was used as a control for potential *S. tragus* seeds already in the soil seedbank. Seedling emergence were counted and removed in all plots on all evaluation dates: March 16, March 31, April 14, April 28, May 12, May 26, June 9, June 23 and July 7 in F/WW1 and SW/F/WW1, and April 7, April 21, May 5, May 19, June 2, June 16, June 30, July 14 and July 28 in F/WW2 and SW/F/WW2, with the exception of ten seedlings per replication and evaluation date. How those ten seedlings were chosen it was by assigning randomly one of the nine evaluation dates to each sprinkled plot (nine per replication). That way, on each evaluation date, the evaluators knew in which plots they had

to count but leave alive ten seedlings. Those ten seedlings were marked to avoid being recounted and removed in following evaluation dates.

Seeds were sprinkled on March 5 and 23, 2020, in F/WW1 and SW/F/WW1, and on March 13 and 10, 2021 in F/WW2 and SW/F/WW2. Seeds for F/WW1 and SW/F/WW1 in 2020 were collected in October 2019 from *S. tragus* plants in a grower's field south of Ione, OR (45.3862°N; 119.8436°W). Seeds for F/WW2 and SW/F/WW2 in 2021 were collected in October 2020 from a different grower's field south of Ione, OR (45.4415°N; 119.8788°W). The viability of seeds collected in fall 2019 and 2020 was 86% and 58%, respectively. After sprinkling *S. tragus* seeds, spring wheat (cultivar Ryan) was planted on SW/F/WW1 and SW/F/WW2 using a no-till drill (Great Plains 606NT, Salina, KS, USA) with 25 cm inter-row spacing, at a seeding rate of 120 kg ha⁻¹ (Table 1). All subsites were fenced for the duration of the experiment to prevent *S. tragus* plants from dispersing additional seeds throughout the experimental area. All plots were hand-weeded. No postemergence or residual herbicides were applied that could affect the emergence of *S. tragus*.

The soil at CBARC is a Walla Walla silt loam (8% clay, 27% sand, and 65% silt) with 2.3% organic matter and pH of 5.4. Long-term average precipitation at CBARC is 420 mm per year (Williams et al. 2020). Spring and winter wheat crops were managed following common no-till practices in the area. Fertilization and pest management practices were applied in all sites following standard recommendations (Wysocki et al. 2007). Precipitation and average daily air temperatures were obtained from a local weather station at CBARC (Figure 1).

Seedling emergence and seedbank persistence

Emerged *S. tragus* seedlings were counted and removed approximately every 14 days from March to July in all plots during the first year of each subsite. However, in subsequent years, seedlings were only recorded in four plots per replication, the control plots and the three plots with the latest assigned emergence dates. This was because *S. tragus* plants in these plots were killed by freezing temperatures in October before they could produce viable seeds.

To consider the presence of seeds in the seedbank prior to the experiment, the *S. tragus* emergence observed in the control plots (which had not received any seeds and therefore represented the natural seedbank) were averaged per site and used to correct the counted

emergence in the other plots. The correction consisted in subtracting the average emergence observed in the control plots from the emergence observed in the other plots (sprinkled plots), when a negative value was obtained a zero value was recorded instead. Seedling emergence in percentage per plot was estimated by dividing the number of emerged seedlings per plot by the percentage of seed viability estimated in 400 seeds per year and multiplying the result by 100.

Plant biomass and viable seed production

Plant biomass and viable seed production were recorded during the first year of each subsite. On every *S. tragus* emergence sampling date, in the plot assigned to that sampling date, ten seedlings were marked with a 30 cm wood stick and allowed to grow during subsequent emergence samplings dates. Starting in mid-August, one plant per plot was harvested weekly. Harvested plants were bagged individually and weighed. Plants were dried in an unheated greenhouse until a constant dry weight was achieved.

The total number of seeds per plant was determined by threshing to obtain all seeds, then weighing a one-gram subsample of all threshed material and counting all filled seeds in the subsample. Seed viability was evaluated using germination tests in a germination chamber. The number of viable seeds was estimated based on the total number of seeds per plant and the seed germination percentage observed in the germination tests.

Seed germination tests

Salsola tragus seeds were stored at room temperature until germination tests were performed. In 2020, fifty seeds per harvest date were placed in plastic boxes with blotter paper moistened with 10 ml of water. In 2021, twenty-five seeds per plant and harvest date were germinated in the same manner. Seeds were incubated at 25 °C with a 12 h photoperiod for 28 days in a germination chamber. All germination tests were repeated twice. Germination was checked and recorded every other day. Water was added to the boxes if needed.

Statistical analysis

Salsola tragus seedling emergence was analyzed with a mixed, nested ANOVA. The statistical model included year and cropping system as fixed effects, and subsite nested within year as a random effect. The random effect accounted for variability among subsites within each year,

recognizing that different locations might have unique characteristics impacting seedling emergence. Seedbank persistence in the two cropping systems was analyzed using a two-way ANOVA, with cropping system (site) and the chronological year as fixed factors. Plant biomass and number of viable seeds were analyzed with a multi-way ANOVA with year, emergence date, harvest date, and cropping system as fixed effects.

In all ANOVA analyses, the normality of data distribution and homogeneity of variance assumptions were tested using Levene's test and Shapiro-Wilk test. When these assumptions were not met, a square-root-arcsine transformation was applied to normalize the data. If the assumptions remained unmet, the generalized least squares (GLS) method with adjustments for heterogeneity of variances was employed. The significance of the effects was evaluated using F-tests, with p-values adjusted for multiple comparisons. Post-hoc comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test at 0.05 significance level to identify specific differences between factor levels, utilizing the nlme package (Pinheiro et al. 2018). Analyses were performed with R version 4.3.1 (R Core Team 2023).

Gompert, Weibull, and logistic regression models commonly used to describe weed emergence (Gonzalez-Andujar et al. 2016), were fitted to the cumulative seedling emergence data. The logistic model provided the best fit to our data. This model is described in Equation 1 and the estimated parameters in Table S1:

$$E (\%) = \frac{a}{1 + e^{-\left[\frac{x - t_{E50}}{b}\right]}} \text{ Eq. 1}$$

where E is the cumulative emergence over time, a is the maximum emergence, x is the time in days, t_{E50} is the time to reach 50% final emergence (days), and b indicates the slope around t_{E50} . The regression model, fitted using GraphPad Prism 6 (GraphPad Software, Inc.), was used to calculate the number of days required to reach 10% and 50% of seedlings emerged for each experiment.

Results and Discussion

Weather data

Accumulated precipitation was 174 mm in 2020, 62 mm in 2021, 225 mm in 2022, and 72 mm in 2023 from mid-March to mid-July. Additionally, temperatures during June and July were

approximately 5 °C warmer in 2021 than in 2020, 2022 and 2023 (Figure 1). The accumulated precipitation during the *S. tragus* growing season, from March to September, was 44% greater in 2020 (195 mm) compared to 2021 (85 mm).

Seedbank persistence and seedling emergence.

Salsola tragus seeds sprinkled in 2020 produced seedlings in both cropping systems (F/WW and SW/F/WW) for four years, indicating that seeds in the soil seedbank remained viable for at least four years. However, emergence during the fourth year was very low (0.25% in F/WW1 and 0.46% in SW/F/WW1), and data from the final year were excluded from the analysis.

Considering the first three years after seeding, total seedling emergence was 23.6% greater ($p = 0.003$) in the spring wheat – fallow – winter wheat cropping system compared to the fallow – winter wheat system (47.2% vs 23.6% respectively). Annual seedling emergence was greater ($p < 0.001$) in the first year (71.8%, 32.3%, and 19.2% in F/WW1, SW/F/WW1 and SW/F/WW2, respectively) compared to the second and third years (Figure 2). An exception was observed in F/WW2, where emergence was 6.9% in the first year and 5.6% in the second year.

Soil seedbanks are classified into three types based on the presence of viable seeds in the soil. Species forming transient seedbanks produce seeds that will not remain viable in soil after one year. In contrast, short-term persistent and long-term persistent seedbanks are defined by species which produce seeds remaining viable in soil for 1-3 years or greater than 5 years, respectively (Thompson et al. 1997, Walck et al. 2005). Previous research with *S. tragus* indicated that this species formed transient or short-term persistent seedbanks. Burnside et al. (1996) demonstrated that *S. tragus* seeds buried 20 cm deep in the soil profile remained viable for one year, while Ogg and Dawson (1984) demonstrated that seeds tilled into the upper 5 cm of soil and irrigated continued to emerge after two years.

Our work was performed with no-till farming practices where seeds on the soil surface are more exposed to harsh biotic and abiotic factors including extreme temperatures, abrupt humidity changes (Moyer et al. 1994; Anderson 2005), and seed predation compared to buried seeds (Hulme 1998). Our findings largely agree with Ogg and Dawson (1984), confirming that *S. tragus* most likely forms short-term persistent seedbanks in no-till systems in the PNW. Additionally, more seedling emergence in the spring wheat-fallow-winter wheat rotation compared to the fallow-winter wheat rotation could be attributed to the spring wheat seeding,

which provided better soil-seed contact at the time when temperatures are becoming favorable for *S. tragus* germination. Furthermore, seeds in fallow are more exposed to predation than those in spring wheat (Kalin 2022), which could have contributed to the reduced emergence observed.

The onset date of seedling emergence, defined as when 10% of total emergence occurs, ranged between March 30 and April 6 in F/WW1, SW/F/WW1 and F/WW2 during the first two years (Figure 3). However, seedling emergence was delayed for the third year until May 22, May 4, and April 19, respectively (Figure 3, Table 2). In SW/F/WW2 was slightly different, with emergence occurring on March 23 in the first year and May 23 and April 19 in the second and third years, respectively (Figure 3, Table 2). A similar pattern was observed for the time to reach 50% seedling emergence. Timing was similar in the first and second years for F/WW1 (April 23 and 20), SW/F/WW1 (April 21 and 15), and F/WW2 (April 29 and May 1), but there was a delay of 11 to 40 days in these sites during the third year (Table 2). However, SW/F/WW2 exhibited a slight variation, 50% emergence was observed on April 16 in the first year but was delayed in the second and third year to June 5 and May 12, respectively. These results from SW/F/WW2 align with those of Ogg and Dawson (1984), who noted that seedlings emerged earlier in the first year compared to the second year. However, most of our results indicated a germination delay in the third year for F/WW1, SW/F/WW1 and F/WW2.

Salsola tragus emerged until the end of June and early of July, consistent with a study conducted in dryland crops in a low precipitation region (Lind, WA) (Young 1986). However, Young (1986) related the end of *S. tragus* emergence to the field situation (fallow, spring wheat, or winter wheat), with emergence extending until late June in spring wheat and until mid-July in fallow. In our study, the presence of crops (winter or spring wheat) did not affect *S. tragus* emergence dynamics compared to fallow fields. Additionally, the emergence time reported in our work was later than in observations from a study conducted in an irrigated fallow field in Prosser, WA, where no *S. tragus* emergence was noted after May 1 (Ogg and Dawson 1984).

Plant biomass and viable seed production.

No interactions among year, harvest date, or cropping system were observed nor differences in the main effect of harvest dates on biomass per plant. However, we found an interaction between cropping system and emergence date ($p < 0.001$) and differences between the main effects of cropping system, year, and emergence date. With regards to cropping system, *S. tragus* plant

biomass was 4.4 times greater ($p < 0.001$) in fallow (466 g per plant) compared to spring wheat (105 g per plant), regardless of emergence date and year. This finding is consistent with Young (1986), who reported reduced *S. tragus* growth in competition with spring wheat. With regards to the year, *Salsola tragus* plant biomass (418 g per plant) was 1.4 times greater ($p < 0.001$) in 2021 than in 2020 (290 g per plant on average), regardless of the growth environment (fallow or spring wheat) or emergence date. And with regards to emergence date, in general, plants emerging early in the season exhibited greater biomass ($p < 0.05$) than those emerging later, irrespective of the growing environment or year (Figure 4). For *S. tragus* plants growing in competition with wheat, the reduction in biomass occurred earlier (mid-May) compared to those growing in fallow (beginning of June). This earlier reduction in biomass is likely due to increased competition for light, water, and nutrients. By mid-May, wheat was probably close to peak biomass, and the almost canopy closure at that time, intercepting most of the light, together with a great soil resources absorption, probably restricted *S. tragus* development more than when growing in fallow without resource competition.

No interactions were observed among year, emergence date, harvest date, and cropping system with respect to viable seeds per plant ($p > 0.05$), nor were there differences between years 2020 and 2021 ($p = 0.66$). However, differences were observed based on emergence date ($p = 0.029$), harvest date ($p < 0.001$), and cropping system ($p = 0.028$). A greater number of viable seeds were observed in plants growing in fallow compared to those in spring wheat, 1084 vs. 723 viable seeds per plant, respectively. There was a positive linear relationship between seed production and plant biomass, as previously described by Barroso and Genna (2021). The greater biomass observed in plants growing in fallow conditions, without competition, was also reflected in a greater number of seeds per plant.

In general, the number of viable seeds was greatest in plants emerging in mid-May (1,625 seeds). Plants emerging later (June) produced fewer seeds ($p < 0.05$), likely due to a shorter growing period resulting in smaller plants with reduced seed production as noted by Schillinger and Young (2000). Plants that emerged very early (late March) also produced fewer viable seeds (Figure 5a). The number of viable seeds was influenced by both the emergence date and by the harvest date. Seed numbers were very low (around 26 viable seeds per plant) during the early harvest dates (August 11 to September 1; Figure 5b), as plants harvested before the end of

summer had insufficient time to produce seeds. The number of viable seeds increased in September, reaching a maximum on September 29 (3,083 viable seeds per plant).

Agronomic implications.

Salsola tragus can pose a long-term challenge for weed management in dryland cropping systems of the PNW due to seedbank persistence of at least four years. Growers must pay careful attention to fields for growing infestations, likely the result of emergence following the first year of dispersal, and continue to monitor fields following herbicide applications for seeds emerging after the first year.

Delayed emergence and reduced seedling emergence in subsequent years underline the importance of timely weed control measures, especially in the first year after seed dispersal. This can be addressed through the application of pre-emergence herbicides before or shortly after crop planting, or by optimizing post-emergence herbicide applications to target seedlings using emergence models (Oreja et al. 2024). The study also emphasizes the impact of environmental conditions, such as precipitation and temperature, on *S. tragus* emergence and growth.

Furthermore, competition with spring wheat reduced biomass and viable seed production compared to those in fallow, suggesting that crop competition is an effective strategy for suppressing *S. tragus* growth. However, winter wheat seemed to be a more effective competitor than spring wheat as spring wheat seeding may favor *S. tragus* germination. Plants emerging from April to mid-May exhibited more biomass and fecundity, indicating that management practices should target these cohorts to reduce overall weed pressure and seedbank replenishment. Additionally, controlling plants before September in post-harvest or fallow fields is crucial to minimize the reintroduction of seeds into the soil seedbank.

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Conflicts of interest

The authors declare no conflicts of interest.

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Table 1. Crop rotations, planting dates, and cultivars in all subsites and years. The crop in each column indicates the year of harvest.

Subsite	Management	Year			
		2020	2021	2022	2023
F/WW1	Phase	Fallow	Winter wheat	Fallow	Winter wheat
	Planting date	-	10/14/2020	-	10/12/2022
	Cultivar	-	Bobtail	-	0755
SW/F/WW1	Phase	Spring wheat	Fallow	Winter wheat	Spring wheat
	Planting date	03/24/2020	-	10/07/21	04/06/2023
	Cultivar	Ryan	-	ORCF	Ryan
F/WW2	Phase	-	Fallow	Winter wheat	Fallow
	Planting date	-	-	10/18/2021	-
	Cultivar	-	-	ORCF 2X2	-
SW/F/WW2	Phase	-	Spring wheat	Fallow	Winter wheat
	Planting date	-	03/11/2021	-	10/03/2022
	Cultivar	-	Ryan	-	Bobtail

Table 2. Predicted calendar days to 10% and 50% cumulative emergence from March 15 in the first, second, and third years in all subsites.

Site	Cumulative emergence	First year	Second year	Third year
F/WW1	10%	18	16	69
	50%	39	37	77
SW/F/WW1	10%	17	16	51
	50%	37	31	52
F/WW2	10%	22	21	35
	50%	45	47	59
SW/F/WW2	10%	11	70	35
	50%	32	82	58

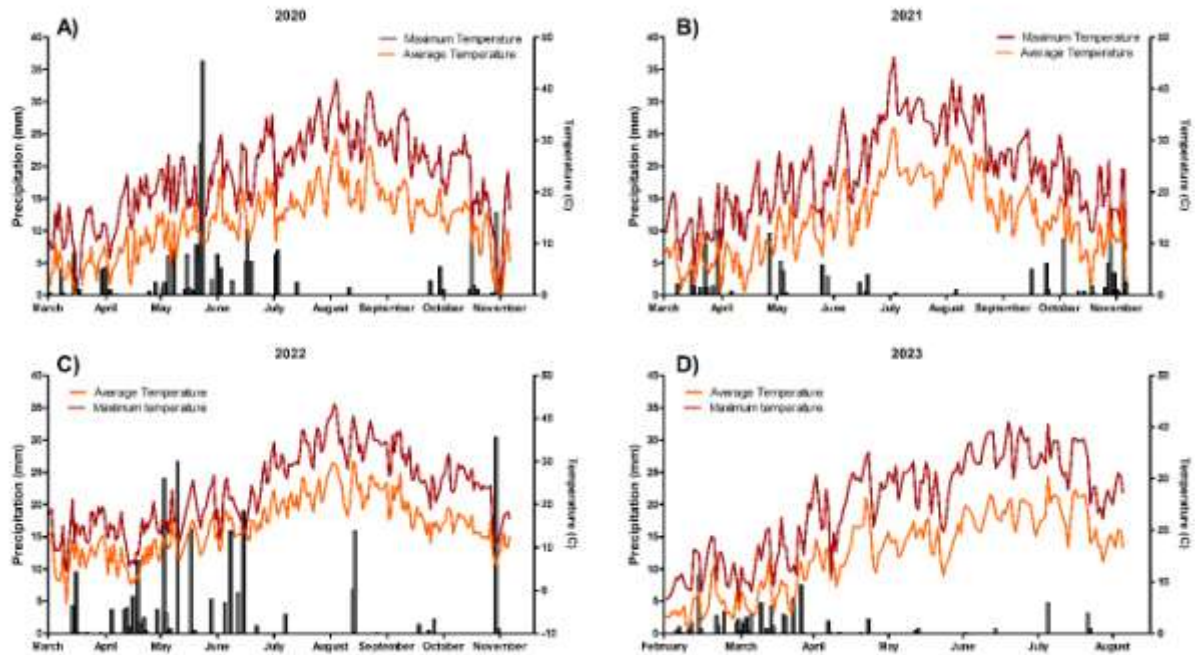


Figure 1. Average and maximum daily air temperature (lines) and precipitation (vertical bars) from March to November in A) 2020, B) 2021, C) 2022 and D) 2023 at the Columbia Basin Agricultural Research Center, Adams, OR.

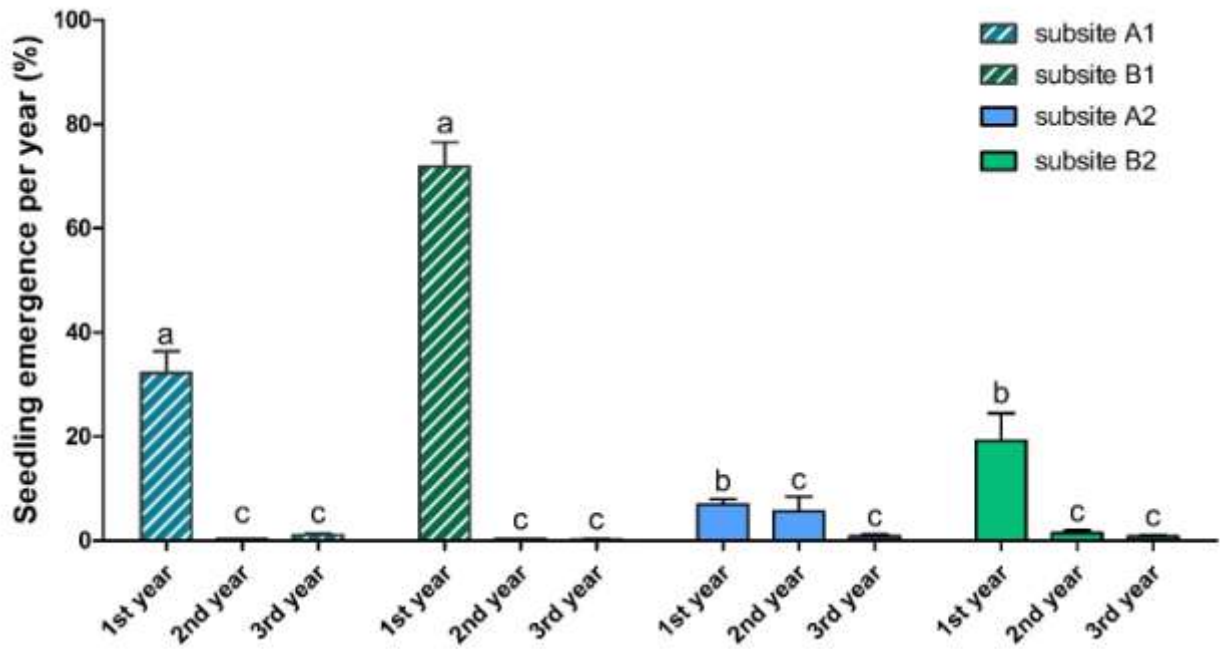


Figure 2. Mean percentage of seedling emergence registered for four years in F/WW and SW/F/WW in the first, second and third year after seeds were sprinkled. Bars indicate the means and whiskers indicate the standard error of the mean (SEM). Different letters on bars mean treatments were significantly different, according to Tukey's multiple comparison test ($p < 0.05$).

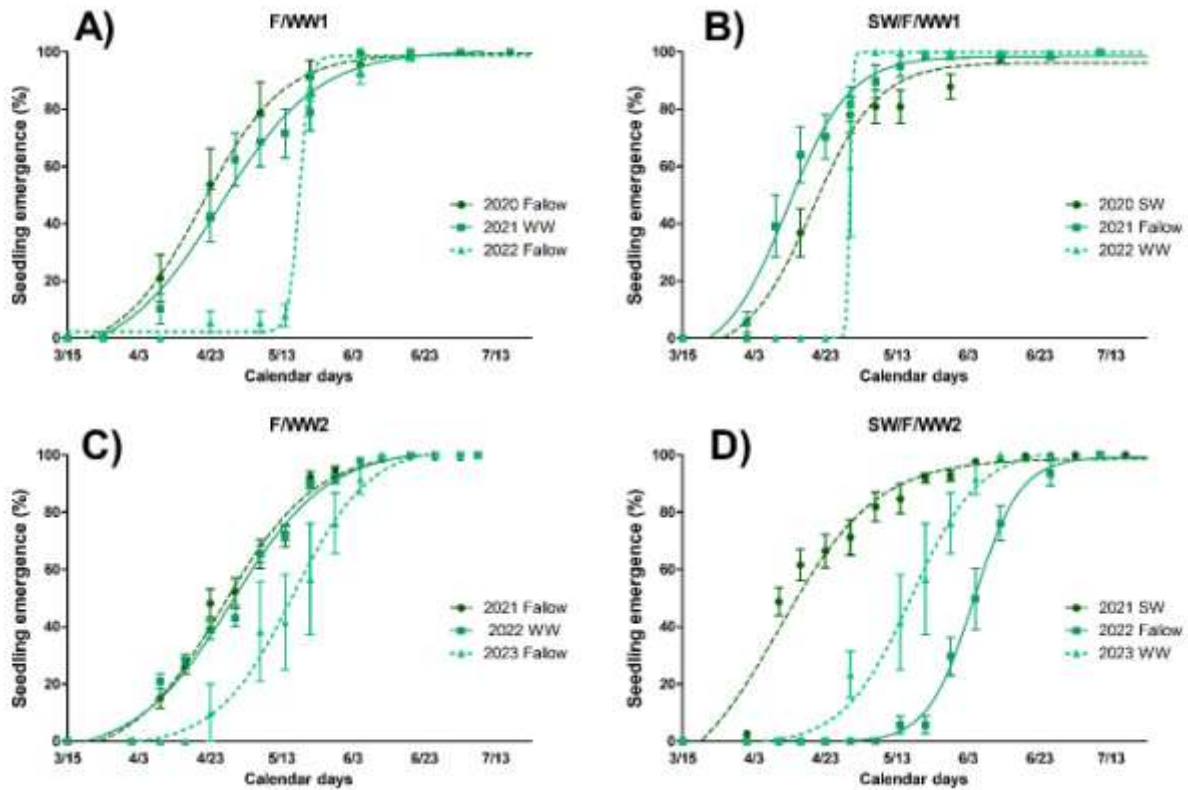


Figure 3. Cumulative seedling emergence registered for the first three years in A) F/WW1, B) SW/F/WW1, C) F/WW2 and D) SW/F/WW2. Markers (circles, squares, and triangles) indicate the means at different emerging dates and vertical whiskers are the standard error of the mean (SEM).

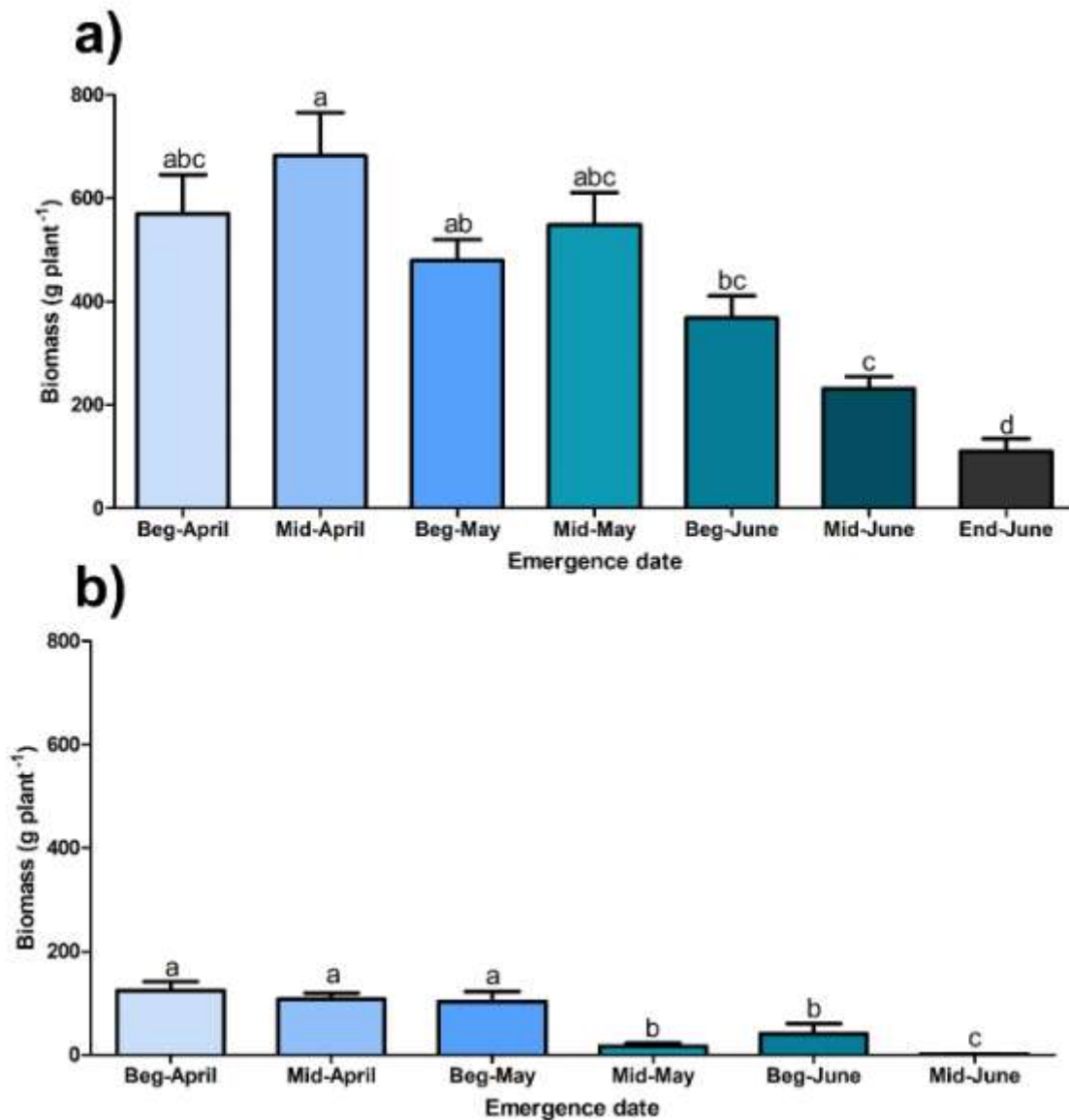


Figure 4. Plant biomass per emergence date (g) in the first year of each cropping system for: a) F/WW (fallow; average between F/WW1 and F/WW2, and harvest dates) and b) SW/F/WW (spring wheat; average between SW/F/WW1 and SW/F/WW2, and harvest dates). Bars indicate the means and whiskers indicate the standard error of the mean (SEM). Bars with different letters indicate differences among emerging dates at each cropping system according to Tukey's multiple comparison test ($p < 0.05$).

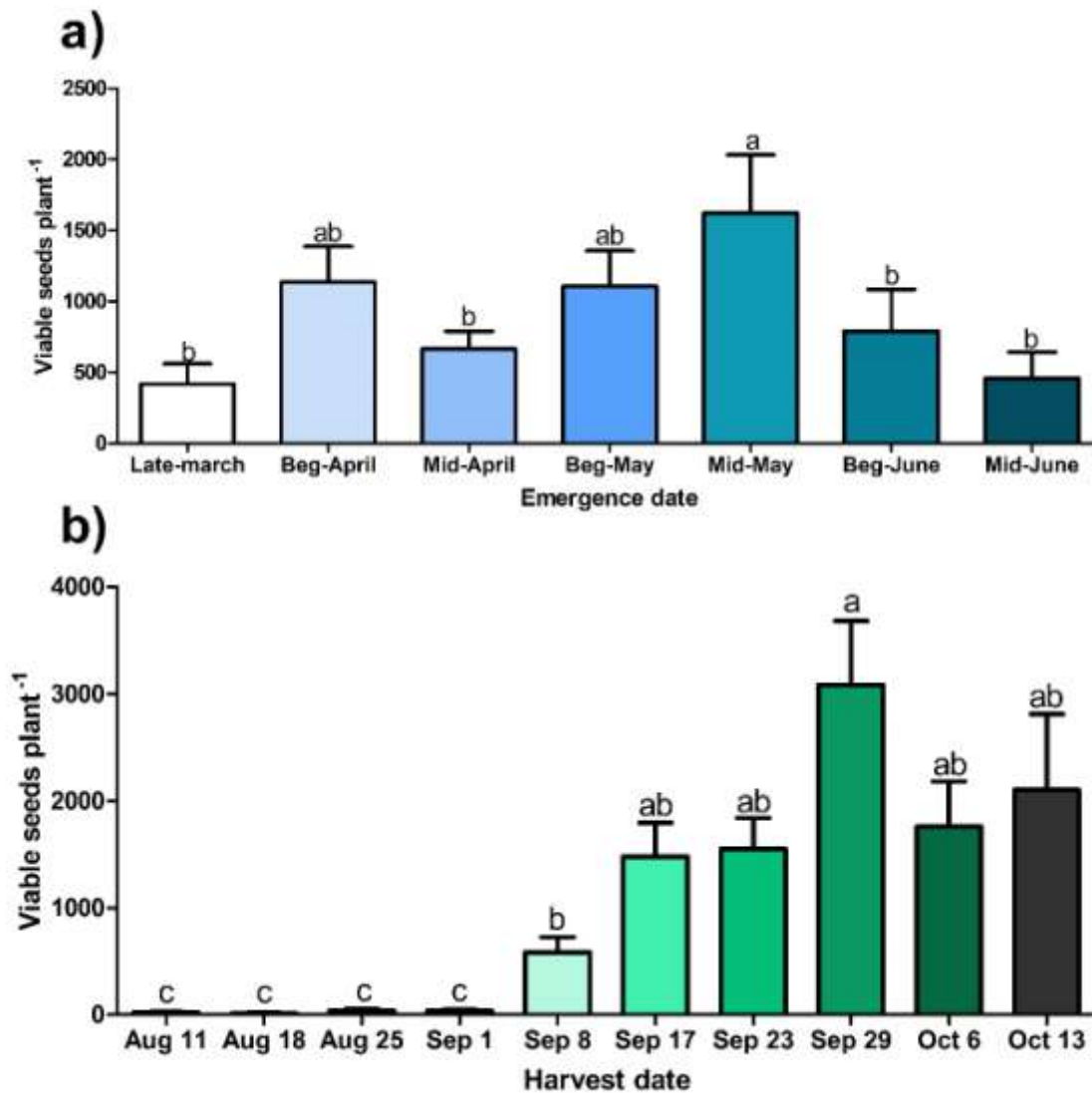


Figure 5. Viable seeds per plant and per emergence date (a) and per harvest date (b), in the first year of both cropping systems and years combined. Bars indicate the means and whiskers indicate the standard error of the mean (SEM). Different letters on the colored bars indicate differences among dates according to Tukey's multiple comparison test ($p < 0.05$).