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Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) Seed Shatter in Wheat

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Abstract

Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] has become a major annual weed in wheat (*Triticum aestivum* L.) production systems in the inland Pacific Northwest. With large genetic variability and abundant seed production, *L. perenne* ssp. *multiflorum* has developed globally 74 documented cases of herbicide resistance covering eight different mechanisms of action. Harvest weed seed control (HWSC) systems were introduced in Australia in response to the widespread evolution of multiple herbicide resistance in rigid ryegrass (*Lolium rigidum* L.) and wild radish (*Raphanus raphanistrum* L.). The efficacy of these systems for any given weed species is directly related to the proportion of total seed retained by that species at harvest time. From 2017-2020, ten *L. perenne* ssp. *multiflorum* plants were collected from three different slope aspects at each location. Collections were initiated in each field when it was visually apparent that seed fill was nearly complete, and seed shatter had not yet occurred. Collection continued at near weekly intervals until the fields were harvested. The number of filled florets on a spikelet was used to assess the degree of seed shatter over time. Seed shatter at harvest was 67% of the total number of florets on each spikelet. Seed shatter was closely aligned with wheat kernel development in both spring and winter wheat. The high percentage of *L. perenne* ssp. *multiflorum* seeds that are shattered by harvest may make HWSC less effective than for *L. rigidum* in Australia; however, seeds with the greatest biomass tend to not shatter prior to harvest, which may increase the efficacy of HWSC for managing the soil seedbank. Strategies like planting earlier maturing wheat cultivars could help HWSC be more effective by having wheat harvest begin earlier when more *L. perenne* ssp. *multiflorum* seeds are still on the mother plant.

Key words: Harvest weed seed control; seed retention

Introduction

Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] is a cool season grass that has become a major annual weed in wheat (*Triticum aestivum* L.) production systems in the inland Pacific Northwest and the Southeastern United States (Hulting et al. 2012; Liu et al. 2016; Stone et al. 1999). *Lolium perenne* ssp. *multiflorum* is competitive with winter wheat for nutrients, water, space, and light (Carson et al. 1999; Hashem et al. 1998). Winter wheat grain yield was reduced by up to 60% in Western Oregon at a *L. perenne* ssp. *multiflorum* density of 93 plants m^{-2} (Appleby et al. 1976). In North Carolina, wheat grain yields were reduced 4.2% for every 10 *L. perenne* ssp. *multiflorum* plants m^{-2} up to 100 plants m^{-2} (Liebl and Worsham 1987). Stone et al. (1999) used 11 data sets (two from North Carolina, three from Texas, and six from Oregon) to determine wheat yield loss from *L. perenne* ssp. *multiflorum* interference. Across these diverse environments, a linear model with a slope of 1.15 times the percentage of *L. perenne* ssp. *multiflorum* plants in the total plant population best predicted yield loss (R^2 = 0.88). Economic losses from *L. perenne* ssp. *multiflorum* infestations in wheat result from competition, increased crop lodging prior to harvest, reduced grain quality and increased dockage (Hulting et al. 2012).

Lolium perenne ssp. *multiflorum*, also known as common annual ryegrass, may have originated as an early cultivar of perennial ryegrass (*Lolium perenne* L.) in European agriculture. *Lolium perenne* ssp. *multiflorum* is a cross-pollinating species that is self-incompatible (Nelson et al. 1997). *Lolium perenne* ssp. *multiflorum* readily hybridizes with *L. perenne* L., resulting in plant populations that are difficult to categorize as either species (DiTomaso et al. 2013). *Lolium perenne* ssp. *multiflorum* plants can be annual, biennial, or a short-lived perennial. There is extensive genetic variability within *L. perenne* ssp. *multiflorum* populations, which has allowed it to adapt to a wide range of environmental conditions (Nelson et al. 1997). This large genetic variability, combined with abundant seed production, widespread use as a pasture grass, a component of some turfgrass seed mixtures, and used in soil erosion control plantings makes *L. perenne* ssp. *multiflorum* an excellent candidate species for the evolution of herbicide resistance (Bobadilla et al 2021; Liu et al. 2016; Rauch et al. 2010). In 2024, there were 74 documented cases of herbicide resistance in *L. perenne* ssp. *multiflorum*, covering eight different mechanisms of action (Heap 2024). Biotypes exhibiting multiple herbicide resistance and involving both target site and nontarget site mechanisms add to the complexity of managing *L. perenne* ssp. *multiflorum* (Liu et al. 2016; Tehranchian et al. 2019).

Lolium perenne ssp. *multiflorum* seed usually germinates in the fall but can germinate any time of year under favorable conditions (DiTomaso et al. 2013). *Lolium perenne* ssp. *multiflorum* seed viability declined rapidly in a well-drained soil, with mean seed viability in the second year of <10% across burial depths from 2.6 to 17.8 cm (Rampton and Chang 1970). However, in poorly drained soil, mean seed viability did not drop below 10% until the fourth year of the study, and a small amount (0.2%) of seed remained viable into the seventh year.

Harvest weed seed control (HWSC) systems were introduced in Australia in response to the widespread evolution of resistance to multiple herbicide classes in rigid ryegrass (*Lolium rigidum* L.) and wild radish (*Raphanus raphanistrum* L.) (Walsh et al. 2013). These systems interrupt the process of establishing or replenishing viable weed seed banks in the soil by targeting mature weed seed at harvest. The efficacy of these systems for any given weed species is directly related to the proportion of total seed retained by that species at harvest time (Walsh and Powles 2014; Walsh et al. 2013). High seed retention rates at harvest for *L. rigidum* (85%) and *R. raphanistrum* (99%) suggest HWSC systems will be effective at reducing seed bank replenishment of these two weed species in Australia (Walsh and Powles 2014).

Windrow burning, a popular HWSC system in Australia, reduced *L. perenne* ssp. *multiflorum* seedling emergence in Eastern Washington to just 1% compared to 63 and 48% for the non-burned check and burned standing stubble treatments, respectively (Lyon et al. 2016). However, this study used seed collected elsewhere and did not investigate seed retention at harvest. Concerns with fire escapes and air quality have limited the use of windrow burning in the Pacific Northwest. San Martin et al. (2021) reported mean seed retention rate for *L. perenne* ssp. *multiflorum* at harvest from six site-years in Eastern Washington of 41% and ranging between 29 and 48%. However, they did not report the details of seed shattering.

The initial objective of this research was to investigate the potential of HWSC for managing *L. perenne* ssp. *multiflorum* by 1) documenting the timing of *L. perenne* ssp. *multiflorum* plants reaching 50% seed shatter in relation to wheat harvest, 2) evaluating *L. perenne* ssp. *multiflorum* seed shatter in wheat in relation to field location, and 3) assessing the weight of seed remaining in the head at harvest vs. seed that is shattered prior to harvest. In this study, seed weight is used as a proxy for seed germinability and early seedling vigor. Following two years of the study in winter wheat, the question arose as to what *L. perenne* ssp. *multiflorum* seed shatter might look like in spring wheat, so these same objectives were applied to spring wheat and two more years of the study were conducted.

Materials and Methods

Site Descriptions

Lolium perenne ssp. *multiflorum* plants were collected from farms growing winter wheat in 2017, 2018, and 2019, and spring wheat in 2019 and 2020 (Table 1.). All farms were located within 15 km of Pullman, WA in the Palouse geographic region and contained *L. perenne* ssp. *multiflorum* populations of varying density. Because of the undulating topography of the Palouse landscape, three subsites were identified in each field based on slope position and were revisited at each collection time. Slope aspect positions with east, northeast, or north exposures (NE) received potentially less solar radiation and were likely slightly cooler during the growing period than other slope positions, while slopes with west, southwest, or south exposures (SW) received potentially greater solar radiation and were likely warmer (Tian et al. 2001). Draw bottoms (B) were potentially intermediate in received solar radiation and likely had more soil water than the other slope positions. Soil type at all Clark farm fields, the 2017 Cook field, the 2018 Cook SW site, and the 2019 Cook field was a Palouse silt loam. The 2018 Cook B and NE sites and the Fleener SW site were a Naff silt loam. The 2019 and 2020 Cook fields and the Fleener NE site were a Thatuna silt loam, and the Fleener B site was a Latah silt loam (USDA-NRCS 2022). All soil types are well-drained silt loams with varying horizon depths. Clark and Fleener farms are privately owned and managed by local growers, whereas the Cook farm is owned by Washington State University and operated as a research farm.

All wheat crops were managed with production practices standard for the area. Winter wheat followed a pulse crop [chickpea (*Cicer arietinum* L.), field pea (*Pisum sativum* L.), or lentil (*Lens culinaris* Medik.)] from the previous year and was seeded each year during the first two weeks of October on each farm. Wheat was direct seeded on Clark and Cook farms and seeded following tillage on the Fleener farm. Winter wheat seeding rates ranged between 135

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and 146 kg ha^{-1} on the Clark and Fleener farms each year, and between 102 and 134 kg ha^{-1} for 2017 and 2018, respectively, on the Cook farm. Clark and Fleener farm winter wheat was fertilized with 168, 33, and 28 kg ha^{-1} of N, P, and S, respectively. Cook farm fields were fertilized at seeding with 102-135, 17-28, and 28 kg ha^{-1} of N, P, and S, respectively. Fertilizer on the Fleener farm was applied through shanks on tillage equipment while fertilizer on the Cook and Clark farms were applied with direct-seed planters at the time of seeding. Spring wheat on the Fleener farm was seeded mid-April 2020 on tilled ground following 2019 winter wheat. On the Cook farm, spring wheat was seeded on tilled ground during the first week of May 2019 and the fourth week of April 2020 following winter wheat. Exact seeding dates are not available on all farms because only ranges are available of when the area of the field sampled was seeded. Spring wheat seeding rates ranged from 134 to 168 kg ha^{-1} while the applied fertilizer range was 112-168, 22-33, and 17-28 kg ha^{-1} of N, P, and S, respectively. Fertilizer on the Fleener farm was applied through shanks on the tillage equipment prior to seeding while fertilizer on the Cook farm was applied with a direct-seed planter at the time of seeding. Fertilizer recommendations for wheat production in this region vary from farm to farm and include soil nutrient status left by previous crop (Koenig 2005). Pyroxasulfone plus carfentrazone-ethyl (Anthem Flex®, FMC Corp., Philadelphia, PA 19104) was applied post-plant by the grower at 11 plus 0.008 g ai ha^{-1}, respectively, to winter wheat on the Clark Albion Rd. and Collings Rd. fields for grass weed control; however, control was not consistent or complete, therefore, *L. perenne* ssp. *multiflorum* could be collected in these fields the following spring. Neither pyroxasulfone or any other preemerge grass herbicides were applied to any other fields in this study (Grower communication). Herbicides applied in spring were to control broadleaf weeds and would not have affected *L. perenne* ssp. *multiflorum*.

Collection Procedures

Ten *L. perenne* ssp. *multiflorum* plants were randomly collected across infested areas at each slope aspect position (NE, B, SW) at each collection time and from each farm and placed individually in paper bags. Collections were initiated in each field when it was visually apparent that seed fill was nearly complete, and seed shatter had not yet occurred. Collection continued at near weekly intervals until the fields were harvested; however, in 2019, collections were made

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only at seed fill and at harvest at each site. All bags were stored in larger grocery-size paper bags at room temperature in a lab with humidity ranging between 35% and 40%, temperature ranging between 20 C to 23 C, and with continual ventilation until winter months when processing occurred. In addition, the maturity of wheat kernels was assessed at each field site and slope aspect position by randomly sampling wheat kernels from different plants at each collection date (Table 2). Wheat kernels were pressed between fingers or teeth to determine their stage of development in correspondence with Feekes growth stages (Large 1954) and expressed as milk stage, Feekes 11.1, soft dough, Feekes 11.2, hard dough, Feekes 11.3, or ripe, Feekes 11.4.

Seed Weight and Shatter Determination

Lolium perenne ssp. *multiflorum* caryopses retain the lemma and palea at disarticulation unless handled roughly, thus for this study we refer to intact florets or caryopses as seeds. The spikelet is the basic unit of an *L. perenne* ssp. *multiflorum* flower and contains a single stem (rachilla) bearing florets. In this study, the number of filled florets on a spikelet is used to assess the degree of seed shatter over time. For each *L. perenne* ssp. *multiflorum* plant, the number of culms and spikelets on each culm were counted. All spikelets were then hand-threshed to carefully separate florets from the rachilla. Beginning with the 2018 collections, all florets from a single unshattered representative spikelet on each plant were counted from the first collection dates to determine the potential seed number per spikelet if all florets had filled. Once all spikelets were threshed, the seeds were cleaned using a seedblower (South Dakota Seed Blower Model 757, Seedburo® Equipment Co., Des Plaines, IL 60018) set to remove only chaff and most unfilled florets. Cleaned seeds from each plant were weighed and then 100 seeds were subsampled and weighed to determine 100-seed weight and single-seed weight. If 100 seeds were not available, as was the case for some plants from the later collection times, the seed weights were determined from the total number of seeds in the collection. Total seed weight was divided by single-seed weight to determine total seeds plant^{-1}, which was then divided by the number of spikelets to find the mean number of seeds spikelet^{-1} (SPS).

Weather Data

Because of the relative proximity to all fields sampled in this study, daily weather data were obtained from the Pullman-Moscow Regional Airport, Pullman, WA 99163. Distances from each field location and the Pullman-Moscow Regional Airport ranged from 14 km for the Clark Parvin Rd. site to 5 km for the Cook Farm. Growing degree days (GDD) were calculated from October 1 of the year prior to the *L. perenne* ssp. *multiflorum* seed sampling for both winter and spring wheat crops to correspond with winter wheat planting and post-harvest *Lolium perenne* ssp. *multiflorum* after-ripening using the following equation:

$$GDD = \sum [(Tmax + Tmin) / 2] - Tbase \quad [1]$$

where Tmax and Tmin are the daily maximum and minimum air temperature, respectively, and Tbase is the base temperature, which is 0 C for this study since *L. perenne* ssp. *multiflorum* is a C3 species able to germinate at temperatures near 0 C (Young et al. 1975). Monthly precipitation and temperature data from October through September for each collection year were obtained from the Pullman-Moscow Regional Airport weather station (Network ID: GHCND:USW00094129).

Statistical Analysis

Dependent effects were SPS, seed weight, number of culms per plant, number of spikelets per culm, and total florets per spikelet. Independent fixed effects were collection year, collection week, collection date, and slope aspect position. Farm location was considered a random effect. The number of culms per plant, spikelets per culm, and total florets per spikelet data were analyzed for each year as descriptive variables using PROC MEANS in SAS® V. 9.4 1M5 (SAS Institute 2023) and presented as means ± standard deviation.

The SPS and seed weight dependent effects were analyzed using PROC GLIMMIX in SAS to determine levels of significance and interactions. In all analyses, the LaPlace method was used for maximum likelihood estimation and the containment method was used to assign degrees of freedom. The SPS and seed weight data satisfied normality and variance assumptions upon visual inspection of the Q-Q plots and frequency histograms of the studentized residuals and by

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examining kurtosis numbers. For SPS, analysis of the full model of collection year*collection week*slope aspect position, collection week was used in place of collection date because collection date was not consistent between collection years. Collections were designated numerically by the collection week they were collected starting with 1 for the first week of sampling. For analysis of data within each collection year, collection date was used as the independent fixed effect for collection periods. For seed weight, crop type*collection week*slope aspect position was used as the full model analysis in PROC GLIMMIX in SAS. Comparisons between least squares means were determined using pair-wise comparisons in PROC GLIMMIX ($p \leq 0.05$).

To determine when 50% shatter had occurred each year, the SPS data were regressed by collection date within each year using PROC REG in SAS. The 50% SPS shatter value is one half of the predicted maximum. The fit of linear and quadratic response curves was tested using the lack-of-fit *F* test ($p \leq 0.05$) in PROC REG. Additionally, adjusted R^2 and p-values were calculated and reported from each regression analysis using PROC REG in SAS.

To determine the relationship between crop maturity and GDD, GDD was used as a predictor for crop maturity indicated by Feekes scale numbers. To compare *L. perenne* ssp. *multiflorum* SPS with GDD, GDD was used as a predictor for *L. perenne* ssp. *multiflorum* SPS. Finally, to compare *L. perenne* ssp. *multiflorum* SPS with crop maturity, Feekes scale numbers were used as a predictor for *L. perenne* ssp. *multiflorum* SPS. All regression analyses were performed using PROC REG in SAS.

All statistical graphics were prepared using SigmaPlot Software V. 15, Grafiti LLC, 405 Waverly St., Palo Alto, CA 94301.

Results and Discussion

Regional weather and soil conditions

Soils in the Palouse are dry in late summer and early fall (Ibrahim and Huggins 2011) due to a regional climate of dry summers (Table 3) in combination with soil water extraction from the previous crop. In addition, air and soil temperatures are declining from summer into the fall

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months (Table 3) and in most years freezing temperatures occur in October and November. Therefore, we have observed that *L. perenne* ssp. *multiflorum* germination in the fall is limited both by available moisture and cold soil temperatures. Furthermore, it has also been observed that fall-germinated *L. perenne* ssp. *multiflorum* sometimes has difficulty surviving through the winter if soils are frozen without snow cover and if the seedlings experience frost heaving (personal observation). In this region, most *L. perenne* ssp. *multiflorum* germination has been observed beginning in March and continuing through May. In this study, collection locations were identified beginning in June of the collection year when *L. perenne* ssp. *multiflorum* culms were visible. In winter wheat, most *L. perenne* ssp. *multiflorum* plants emerged as soil temperatures were warming in late winter and early spring; however, any plants surviving the winter would have been present and well tillered in spring. In spring wheat, all *L. perenne* ssp. *multiflorum* plants emerged after the crop was seeded, as plants that germinated prior to seeding were controlled with tillage, herbicide, or both.

Crop stage and weather

Lolium perenne ssp. *multiflorum* seed collections were initiated each year when it was apparent from visual inspection that seeds were filled, and shattering had not yet begun. All wheat was in the milk stage or early soft dough stage when sampling began (Table 2). In each year, crop stage progressed from milk to ripe stages during the collection period, which ended when the wheat was ripe and harvest began (Table 2). Of the four collection crop years, 2016-17 was the warmest overall and had the highest accumulated GDD relative to calendar dates, but 2017-18 was also relatively warm. In contrast, the crop year with the lowest accumulated GDD was 2019-20. Mean monthly temperatures were most extreme in the 2016-17 crop year with December and January having the coldest temperatures while June, July, and August were the hottest. Precipitation was also greatest for the 2016-17 crop year, which was 92 mm over the 30-year mean for the area (Table 3). The driest crop year was 2019-20, which was 60 mm below the 30-year mean.

The number of reproductive structures on each *L. perenne* ssp. *multiflorum* plant was variable (Figure 1); however, reproductive plants had at least one reproductive culm with a spike-type inflorescence containing alternately arranged spikelets oriented edgewise on the

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rachis. Adventitious branching was seen on some spikes, which is not common but has been reported elsewhere (Maity et al. 2021). Spikelets contained florets alternately arranged on the rachilla, which were awned from the tip of the lemma. In our collections from all four years, the mean number of culms per plant was three to five, the mean number of spikelets per culm was 17 to 20, and the mean number of total florets per spikelet was 11 to 13 (Table 4). The overall mean was 967 seeds per plant with a range of 103 to 2525 (data not shown).

L. perenne ssp. multiflorum seed shatter in winter wheat

Understanding the timing and spatial patterns of seed shatter in *L. perenne* ssp. *multiflorum* is important in developing management practices to maximize effectiveness of HWSC and to minimize seeds going into the soil seedbank. Our findings are similar to those reported by San Martín et al. (2021) for the Palouse Region of eastern Washington that showed a majority of *L. perenne* ssp. *multiflorum* seeds are shattered by the time harvest begins; however, by measuring shatter at the spikelet level, we can better understand when and how seed shatter is occurring, and the implications for management of seeds that shatter early versus those that remain on the plant until harvest.

Initial full-model GLIMMIX analysis found a three-way interaction with collection year*collection week*slope aspect position (p=0.042) as collection week and slope aspect position patterns of *L. perenne* ssp. *multiflorum* seed shatter differed between collection year (Table 5); therefore, data were analyzed separately by collection year for all regression analyses. For analysis by collection year, collection date was used instead of collection week (data not shown). There were no significant or meaningful interactions between collection date and slope aspect position; therefore, the main effects of collection date and slope aspect position are presented.

In 2017, *L. perenne* ssp. *multiflorum* sampling began July 6 as the winter wheat was in the milk stage and the *L. perenne* ssp. *multiflorum* seeds had not visibly begun shattering (Figure 2). *Lolium perenne* ssp. *multiflorum* shatter in 2017 followed a quadratic pattern declining from a maximum of 7.6 SPS on July 6 to 2.7 SPS on August 1 when harvest began. Based on the regression equation, 50% SPS of the maximum seed fill occurred 9 d before harvest. The effect

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of slope aspect position was significant (p=0.007) with both the NE and B positions resulting in 5.1 SPS compared with 4.5 SPS for the SW position.

In 2018, the effect of collection date was modeled beginning with the July 11 sampling date when the winter wheat was in milk to soft dough stage. The regression was best fit with a quadratic equation (Figure 3). The maximum seed fill of 8.4 SPS occurred on July 11 and the minimum of 2.8 SPS occurred on August 6 at harvest. The 50% SPS level of 4.2 occurred 10 d before harvest. The slope aspect position effect was not significant.

In 2019, *L. perenne* ssp. *multiflorum* was collected in winter wheat at seed fill on August 6 and again at harvest on August 18, which yielded a simple estimated linear relationship between sampling days (Figure 4). Winter wheat was at early soft dough stage on August 6 when the first collections were taken. Maximum *L. perenne* ssp. *multiflorum* SPS was 9.3 and declined to 3.2 SPS at the harvest sampling on August 18. The 50% shatter level of 4.7 SPS estimated by the straight line between collection dates occurred 3 d ahead of harvest. Slope aspect position had a significant effect on the number of seeds per spikelet (p=0.003). Both the NE and B positions resulted in 6.4 SPS compared with 5.2 SPS for the SW position.

L. perenne ssp. multiflorum seed shatter in spring wheat

In 2019, *L. perenne* ssp. *multiflorum* was also collected in spring wheat at seed fill on August 6 and at crop harvest on September 4. The interaction between slope aspect position and collection date was significant (p=0.01) but was determined not to be meaningful. The effect of collection date on SPS was modeled by an estimated simple linear relationship (Figure 4). At the initial collection date, spring wheat was in the milk stage and *L. perenne* ssp. *multiflorum* had 5.5 SPS, which declined to 1.7 SPS by the harvest sampling on September 4. The 50% shatter level of 2.7 SPS estimated by the straight line between dates occurred 8 d ahead of harvest. The effect of slope aspect position on SPS was significant (p<0.001). Both the SW and B positions resulted in 3.1 SPS compared with 4.5 SPS for the NE position. This site differed from all other sites in that the B position had fewer SPS than the NE position.

In 2020, the interaction between slope aspect position and collection date was not significant (p=0.17). Sampling of *L. perenne* ssp. *multiflorum* began on July 27 when wheat was in milk to soft dough stage (Figure 5). A quadratic regression best fit the data as the maximum SPS of 8.4 occurred by the July 27 sampling and then declined to 2.5 SPS by the August 18 harvest collection date. The 50% shatter point of 4.2 SPS occurred 12 d before harvest on August 6. The effect of slope aspect position on SPS was significant ($p<0.001$). Both the NE and B positions had 5.2 SPS compared with 4.0 SPS for the SW position.

In all years and in both winter and spring wheat, *L. perenne* ssp. *multiflorum* seed shatter followed a similar pattern. Maximum *L. perenne* ssp. *multiflorum* seed fill occurred when the wheat was in the milk to soft dough stage and was lowest near crop harvest (Figures 2-5). Maximum SPS was 7.8 and the minimum SPS was 2.6. At harvest, 33% of the seeds were still on the plant; therefore, seed shatter was 67%. This is in strong contrast with <25% shatter at crop harvest reported for *L. rigidum* in Australia (Walsh and Powles 2014) or for *L. perenne* ssp. *multiflorum* in soft red winter wheat in Kentucky (Herman and Legleiter 2023).

Heat accumulation, as measured by GDD, was a reasonable predictor of maturity in winter and spring wheat (Figure 6a, 6b). However, relying on GDD to predict seed shatter was less reliable than using wheat stage (Figure 6c, 6d). The stage of the wheat crop was a slightly better predictor of *L. perenne* ssp. *multiflorum* SPS (Figure 6e, 6f), but there was still a considerable amount of variability. Future studies should utilize weather stations at each farm or dataloggers with temperature sensors at each field for more precise measurement of heat accumulation to better assess whether GDD could be a reliable predictor of seed shatter.

Seed shatter in *L. perenne* L. occurs at an abscission layer located directly beneath each floret, which develops during anthesis; therefore, by heading, the abscission layers are well developed, and by five weeks after anthesis many seeds have shattered, beginning with the uppermost florets in each spikelet (Elsgerma et al. 1988). This is consistent with what we found with *L. perenne* ssp. *multiflorum* where seed shatter began during the week following maximum seed fill and then continued until crop harvest. In *L. perenne* L., the development of the abscission layer is controlled by the expression of at least eight genes (Fu et al. 2018), therefore, it is a trait that can be selected for over time where greater gene expression results in greater or sooner levels of shatter. Development of abscission layers in *L. perenne* ssp. *multiflorum* has not been studied, but the patterns of seed shatter suggests that *L. perenne* ssp. *multiflorum* is more similar to *L. perenne* L. in relation to shatter from abscission layers than with *L. rigidum,* which

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is reluctant to shatter during the crop harvest period (Walsh and Powles 2014). In the Palouse, *L. perenne* ssp. *multiflorum* has been present in crops for at least 30 years (grower and personal communication) but seed shatter timing and rates for earlier populations are unknown. However, the near perfect timing of *L. perenne* ssp. *multiflorum* anthesis and shatter with the timing of wheat development, along with the local climate, suggests there has been selection for *L. perenne* ssp. *multiflorum* populations that corresponds well with wheat crop development and harvest.

In our collections, *L. perenne* ssp. *multiflorum* seed shatter reached the 50% SPS level approximately a week before crop harvest began in both winter and spring wheat. Furthermore, by harvest, the rate of shatter was approaching a minimum level. Overall, in winter wheat, 50% shatter occurred 7 days before the start of harvest. Based on our regression, if harvest had occurred 10 days sooner than it did in 2017 and 2018, 4.2 *L. perenne* ssp. *multiflorum* SPS would have remained on the plants. Overall, in spring wheat, 50% shatter occurred 10 days before the start of harvest. If spring wheat harvest had started 10 days earlier in 2020, 4.2 SPS would have been left on the plants. Overall, slope aspect position had only a minor effect on timing of seed shatter with the SW position having slightly fewer seeds than the NE or B positions; however, a standard practice of some growers is to harvest hilltops and SW slopes first as they often ripen before NE slopes, and this might be a strategy to increase the success of HWSC. The 50% shatter level is an artificial benchmark, but it is a useful reference point for the shatter rate in relation to wheat maturity. Since success of HWSC depends on seeds remaining on the plant at harvest (Soni et al. 2020; San Martín et al. 2021; Walsh et al. 2018; Walsh and Powles 2014), one strategy would be to plant earlier maturing wheat cultivars so that harvest could occur earlier. For winter wheat cultivars grown in the high rainfall region of eastern Washington and northern Idaho, there is approximately an 8-day difference between the longest and shortest days to heading, and for spring wheat cultivars there is a 6-day difference (WSU Small Grains 2023). Shirtliffe et al. (2000) determined that an earlier harvest could be effective in capturing a greater number of wild oat (*Avena fatua* L.) seeds with the combine. Therefore, moving wheat harvest up a few days would capture more *L. perenne* ssp. *multiflorum* seeds in the combine where they could be controlled with HWSC methods.

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Seed weight

Lolium perenne ssp. *multiflorum* seed weight increased with the time seeds remained on the plants in both winter and spring wheat (Figure 7a, 7b). Full-model GLIMMIX analysis of the seed weight data found a three-way interaction between crop type, collection week, and slope aspect position (p=0.001). Therefore, all *L. perenne* ssp. *multiflorum* data were analyzed by crop type; however, the 2019 data were analyzed separately by crop type because of only having two collection weeks for each crop. In the 2017-18 winter wheat, *L. perenne* ssp. *multiflorum* seed weight increased incrementally from the first to the fourth collection week (Figure 7a). In contrast, in the 2020 spring wheat, *L. perenne* ssp. *multiflorum* seed weight only increased between the first and second collection week (Figure 7b). This difference was likely influenced by the timing of *L. perenne* ssp. *multiflorum* germination in each crop. In winter wheat, *L. perenne* ssp. *multiflorum* can germinate and establish in many flushes from early spring forward without being subject to any post-emergence control. In spring wheat, early flushes of *L. perenne* ssp. *multiflorum* would have been controlled prior to wheat seeding; therefore, *L. perenne* ssp. *multiflorum* establishment would have likely been more uniform.

In 2019, *L. perenne* ssp. *multiflorum* seed weight was not different between collection weeks in winter wheat but did increase from seed fill to crop harvest in spring wheat (Figure 8). The lack of difference in winter wheat was likely due to only having three weeks between the two collections, while in spring wheat there were five weeks between collections. If sampling in winter wheat had started sooner, differences in seed weight would have likely been found.

In all collections, the remaining 2 to 3 seeds on the plants at harvest were less likely to shatter and were held tightly in the spikelet by the robust glume at the base of each spikelet (Figure 9; personal observation). *Lolium perenne* ssp. *multiflorum* glumes are shorter than the total spikelet length, but in our collections, contained the bottom two to three florets, which often required a pair of tweezers to extract. For those remaining seeds to fall to the ground, it is likely the entire spike would need to break from the culm. Consequently, the seeds remaining on the plant at harvest had the greatest biomass and potentially more seed energy reserves. In other forage grasses, it has been shown that heavier seeds tend to have greater emergence and mesocotyl plus coleoptile length (Andrews 1997) and produce plants with greater shoot length and biomass (Smith et al. 2003). Even though approximately 67% of *L. perenne* ssp. *multiflorum*

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seeds shatter out on the ground before wheat harvest began, destruction of these more robust seeds with HWSC could potentially increase control in following years.

Our research documents the scale and timing of seed shatter of *L. perenne* ssp. *multiflorum*, in both spring and winter wheat in the Palouse region of eastern Washington and northern Idaho. *Lolium perenne* ssp. *multiflorum* seed shatter begins at the uppermost florets on each spikelet and moves down the rachilla to the lower florets with each floret breaking off the rachilla at the abscission layer. The lowest florets are held more secure by the glume and do not easily shatter; therefore, shatter at harvest was 67% of the total number of florets (2-3 out of ~12) on each spikelet. Seed shatter was closely aligned with wheat kernel development in both spring and winter wheat, regardless of thermal accumulation (GDD) during the crop year. The high percentage of *L. perenne* ssp. *multiflorum* seeds that are shattered by harvest may make HWSC less effective than for *L. rigidum* in Australia; however, seeds with the greatest biomass tend to not shatter prior to harvest, which may increase the efficacy of managing the soil seedbank with HWSC. Although intense reliance on HWSC may select for plants that shatter earlier, strategies like planting earlier maturing wheat cultivars could help HWSC be more effective by having wheat harvest begin earlier when more *L. perenne* ssp. *multiflorum* seeds are still on the mother plant.

Funding

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Competing interests: The authors declare none.

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Table 1. *Lolium perenne* ssp. *multiflorum* collection sites near Pullman, WA.

Year	Farm	Crop	Latitude, longitude	Elevation[a] max, min (m)
2017	Clark Albion Rd.	Winter wheat	46.81°N, 117.23°W	758, 755
2017	Clark Parvin Rd.	Winter wheat	46.85°N, 117.22°W	770, 754
2017	Cook	Winter wheat	46.78°N, 117.09°W	781, 776
2018	Clark Collins Rd.	Winter wheat	46.82°N, 117.20°W	752, 749
2018	Cook	Winter wheat	46.78°N, 117.10°W	798, 794
2019	Fleener	Winter wheat	46.82°N, 117.06°W	807, 805
2019	Cook	Spring wheat	46.78°N, 117.09°W	795, 784
2020	Fleener	Spring wheat	46.82°N, 117.06°W	808, 804
2020	Cook	Spring wheat	46.78°N, 117.09°W	780, 777

[a]Maximum (max) and minimum (min) elevations are within each farm location where *L. perenne* ssp. *multiflorum* plants were collected.

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Table 2. Wheat kernel stages at *Lolium perenne* ssp. *multiflorum* collection dates in winter wheat (WW) and spring wheat (SW) crops at three slope aspect positions, north to east (NE), draw bottoms (B), and south to west (SW) in relation to growing degree days (GDD).

		Collection		Field slope aspect position		
Year	Crop[a]	Date	GDD[b]	NE	B	SW
				------------ wheat kernel stages[c] ------------		
2017	WW	7/6	3697	milk	milk	milk
2017	WW	7/11	3894	milk	milk	milk
2017	WW	7/18	4148	dough	milk-dough	dough
2017	WW	7/27	4488	ripe	dough-ripe	ripe
2017	WW	8/1	4687	ripe	ripe	ripe
2018	WW	7/11	3805	milk-dough	milk-dough	milk-dough
2018	WW	7/18	4082	dough	dough	dough
2018	WW	7/25	4331	dough-ripe	dough-ripe	dough-ripe
2018	WW	8/1	4633	ripe	ripe	ripe
2018	WW	8/6	4811	ripe	ripe	ripe

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2019	WW	8/6	4517	dough	dough	dough
2019	WW	8/18	4958	ripe	ripe	ripe
2019	SW	8/6	4517	milk	milk	milk
2019	SW	9/4	5572	ripe	ripe	ripe
2020	SW	7/27	3976	milk-dough	milk-dough	milk-dough
2020	SW	8/5	4347	milk-dough	dough	dough
2020	SW	8/12	4571	dough-ripe	dough-ripe	dough-ripe
2020	SW	8/18	4821	ripe	dough-ripe	Ripe

[a]Winter wheat in all years was seeded October 1-15; Spring wheat in 2019 was seeded in the first week of May; spring wheat in 2020 was seeded during the third and fourth week of April.

[b]GDD accumulated since October 1 of the previous year.

[c]Wheat kernel stages: milk – milky when squeezed (Feekes 11.1); dough – mealy kernel ranging from soft to hard (Feekes 11.2-11.3); ripe – hard kernel harvestable (Feekes 11.4).

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Table 3. Monthly precipitation (PPT) and mean air temperature (Temp) for cropping years October through September[a].

	--- 2016-17 ---		--- 2017-18 ---		--- 2018-19 ---		--- 2019-20 ---	
Month	PPT	Temp	PPT	Temp	PPT	Temp	PPT	Temp
	mm	C	mm	C	mm	C	mm	C
October	127	10	63	9	15	9	48	7
November	47	8	77	5	54	4	11	4
December	39	-4	85	-2	91	1	44	2
January	38	-6	56	3	49	1	85	2
February	81	1	56	-1	73	-4	75	2
March	133	6	37	5	17	1	30	4
April	49	8	60	8	93	9	15	8
May	51	13	49	15	30	14	99	13
June	25	17	36	16	23	16	43	15
July	1	22	1	21	2	19	3	19
August	2	22	8	20	16	21	1	20
September	17	16	1	14	27	15	4	17
Total								
	610		529		489		458	

[a]Data collected at Pullman-Moscow Regional Airport, Pullman, WA.

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Table 4. Number of *Lolium perenne* ssp. *multiflorum* culms per plant (CPP), spikelets per culm (SPPC), and total florets per spikelet (TFL) in winter wheat (WW) and spring wheat (SW) in each of four years collected near Pullman, WA.

		Lolium perenne ssp. *multiflorum*[a]		
Collection Year	Crop	CPP	SPPC	TFL
		----------------- mean ± standard deviation[b] -----------------		
2017	WW	5 ± 4.3	20 ± 3.6	NA
2018	WW	4 ± 3.1	19 ± 2.8	12 ± 2.9
2019	WW	5 ± 2.4	20 ± 3.1	13 ± 1.9
2019	SW	4 ± 2.4	17 ± 3.5	11 ± 1.9
2020	SW	3 ± 2.5	18 ± 3.1	12 ± 2.3
Mean	All	4.3 ± 3.6	18.9 ± 3.3	11.9 ± 2.5

[a]Means for 450 plants in 2017; 270 plants in 2018; 60 plants in 2019 in each crop; 240 plants in 2020.

[b]Means and standard deviations calculated for entire data set using the MEANS procedure of SAS.

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Table 5. Full model PROC GLIMMIX Type III tests of fixed effects for *Lolium perenne* ssp. *multiflorum* seeds spikelet^{-1} data from 2017 through 2020.

Effect[a]	NumDF[b]	DenDF[b]	F Value	Pr>F
YEAR	3	5	0.99	0.469
WEEK	4	1018	256.41	<0.001
YEAR*WEEK	9	1018	6.43	<0.001
ASP	2	1018	11.37	<0.001
YEAR*ASP	6	1018	2.04	0.057
WEEK*ASP	8	1018	1.25	0.269
YEAR*WEEK*ASP	18	1018	1.65	0.042

[a]YEAR=collection year; WEEK=collection week; ASP=slope aspect position. [b]NumDF=numerator degrees of freedom, DenDF= Denominator degrees of freedom. Degrees of freedom assigned using the containment method with the GLIMMIX procedure of SAS.

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Figure 1. Diversity of *Lolium perenne* ssp. *multiflorum* inflorescences collected from field sites in the Palouse region 2017-20.

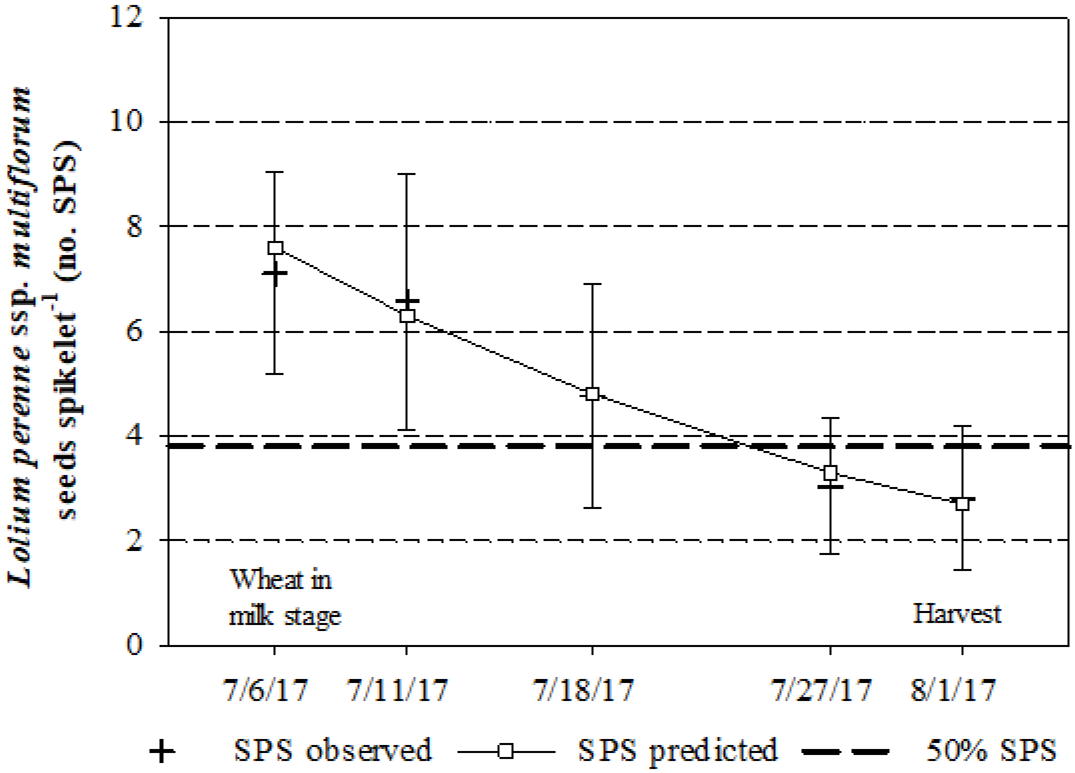

Figure 2. Quadratic regression of *Lolium perenne* ssp. *multiflorum* seed shatter in 2017 winter wheat as the number of seeds per spikelet. Dashed line indicates 50% of the maximum number of seeds per spikelet. Seed shatter reached 50% shatter on 7/23/17. Error bars are standard deviations of the observed means. Regression equation: $y = 0.003x^2 - 0.27x + 7.56$; adj. R^2=0.46; p=0.032.

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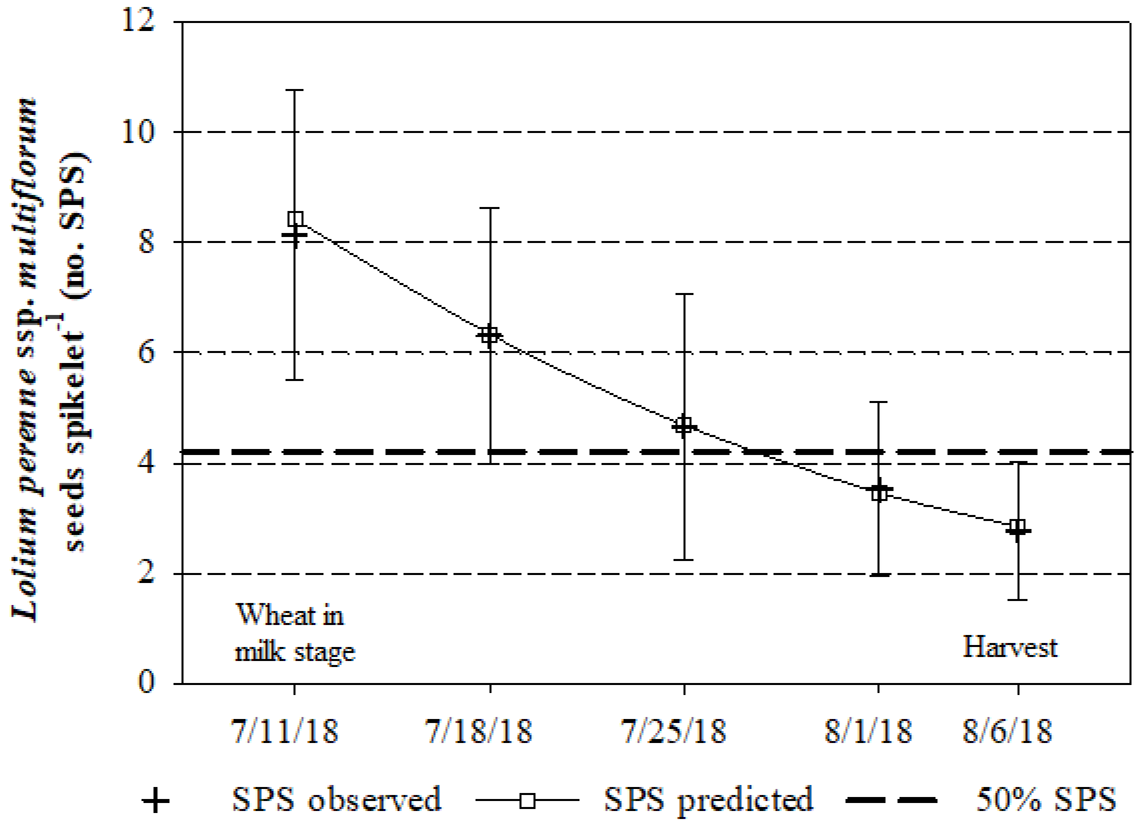

Figure 3. Quadratic regression of *Lolium perenne* ssp. *multiflorum* seed shatter in 2018 winter wheat as the number of seeds per spikelet. Dashed reference line is 50% of the maximum number of seeds per spikelet. Seed shatter reached 50% shatter on 7/27/18. Error bars are standard deviations of the observed means. Regression equation: $y = 0.004x^2 - 0.33x + 8.43$; adj. $R^2 = 0.43$, p=0.049.

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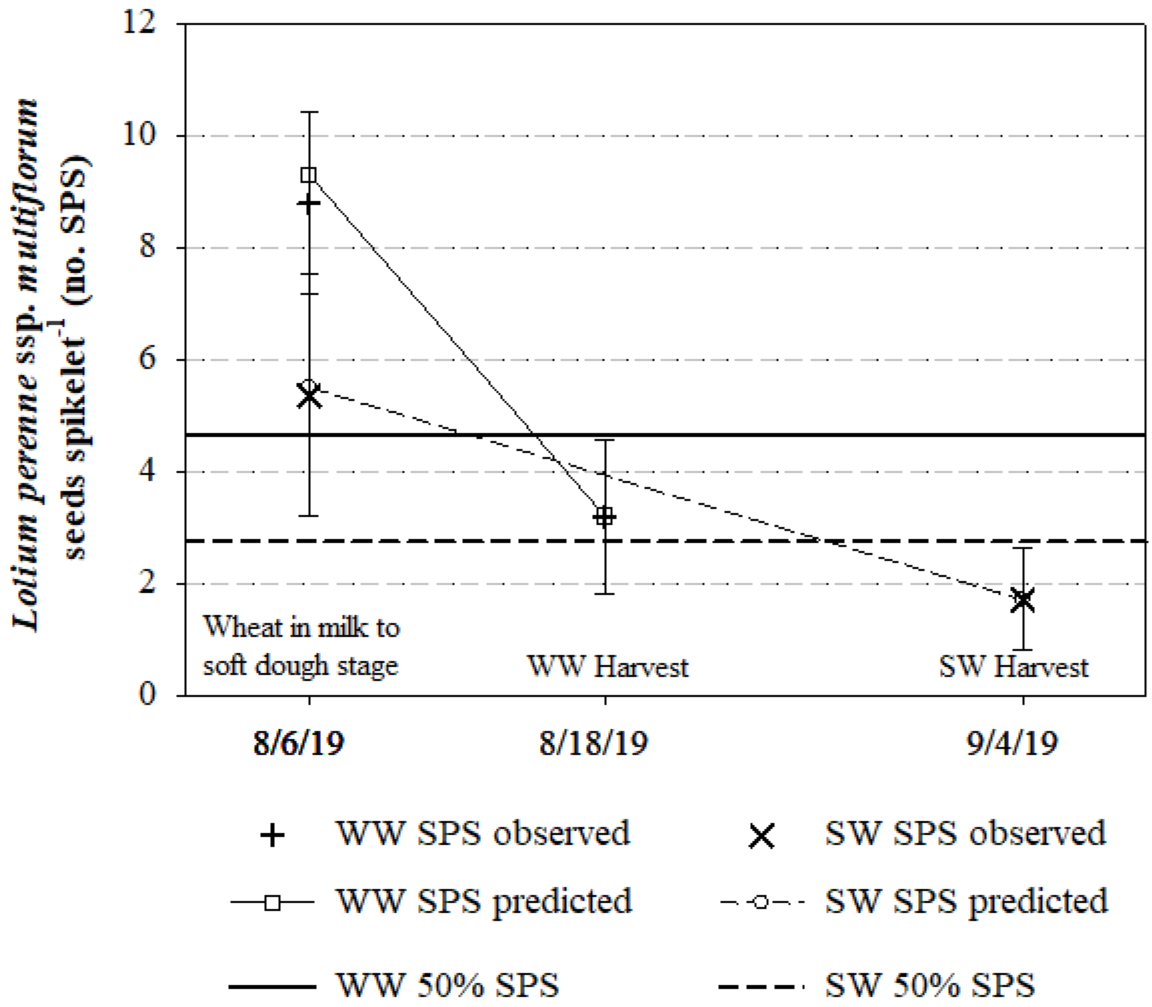

Figure 4. Linear regression of *Lolium perenne* ssp. *multiflorum* seed shatter in 2019 winter wheat (WW) and spring wheat (SW) as the number of seeds per spikelet. Solid reference line is 50% of the maximum number of seeds per spikelet in WW. Dashed line is 50% seeds per spikelet in SW. Seed shatter reached 50% shatter in WW on 8/15/19 and 50% shatter in SW on 8/27/19. Error bars are standard deviations of the observed means. Regression equations: WW y = -0.51x + 9.31; adj R^2 = 0.78, p≤0.001; SW y=-0.13x + 5.48; adj. R^2 = 0.55, p≤0.001.

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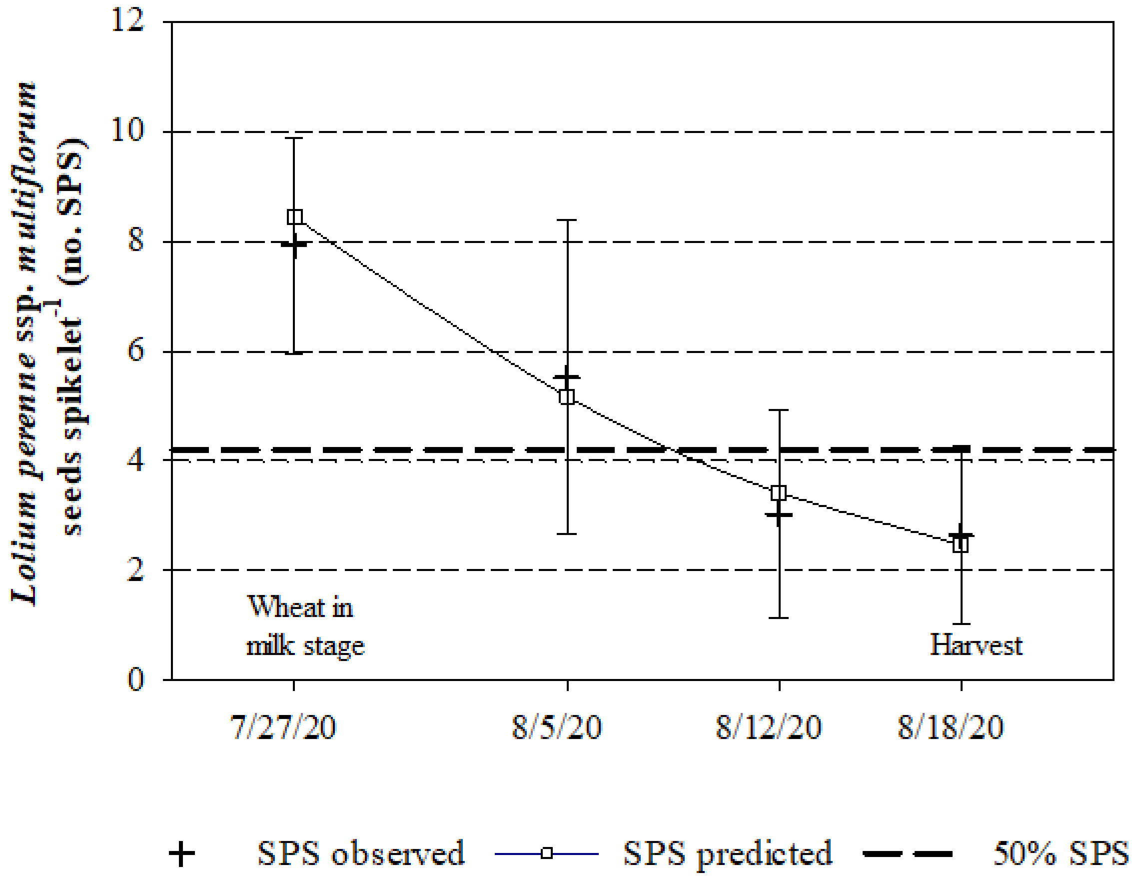

Figure 5. Quadratic regression of *Lolium perenne* ssp. *multiflorum* seed shatter in 2020 spring wheat measured as the number of seeds per spikelet. Dashed reference line is 50% of the maximum number of seeds per spikelet. Seed shatter reached 50% shatter on 8/8/202. Error bars are standard deviations of the observed means. Regression equation: $y = 0.007x^2 - 0.43x + 8.4$; adj. $R^2 = 0.49$, p=0.016.

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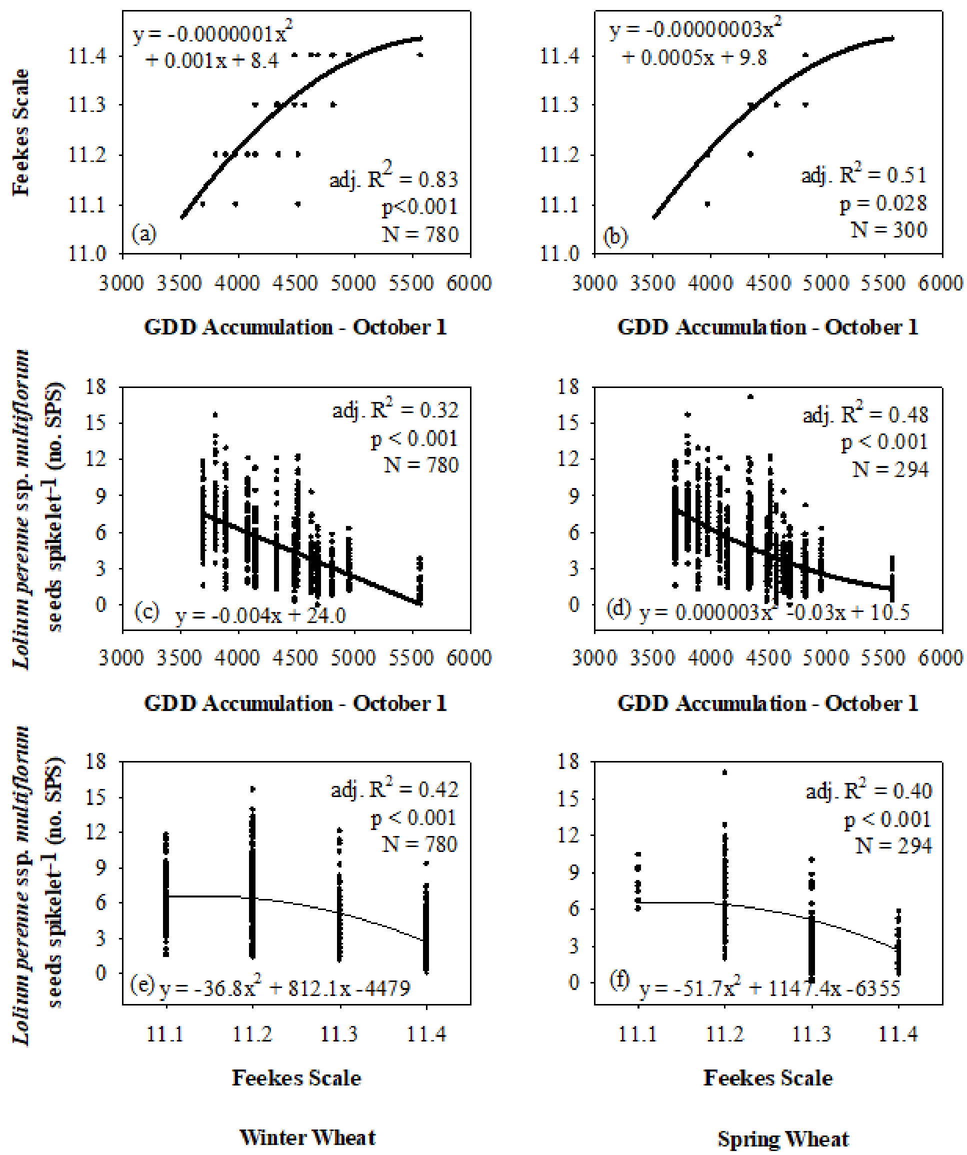

Figure 6. Relationship of winter (6a) and spring (6b) wheat kernel development with growing degree day (GDD) thermal accumulation; relationship of *L. perenne* ssp. *multiflorum* seeds spikelet^{-1} (SPS) collected from winter wheat (6c) and spring wheat (6d) to GDD; relationship of *L. perenne* ssp. *multiflorum* to winter wheat (6e) and spring wheat (6f) kernel development. Regression analysis performed with the REG procedure of SAS.

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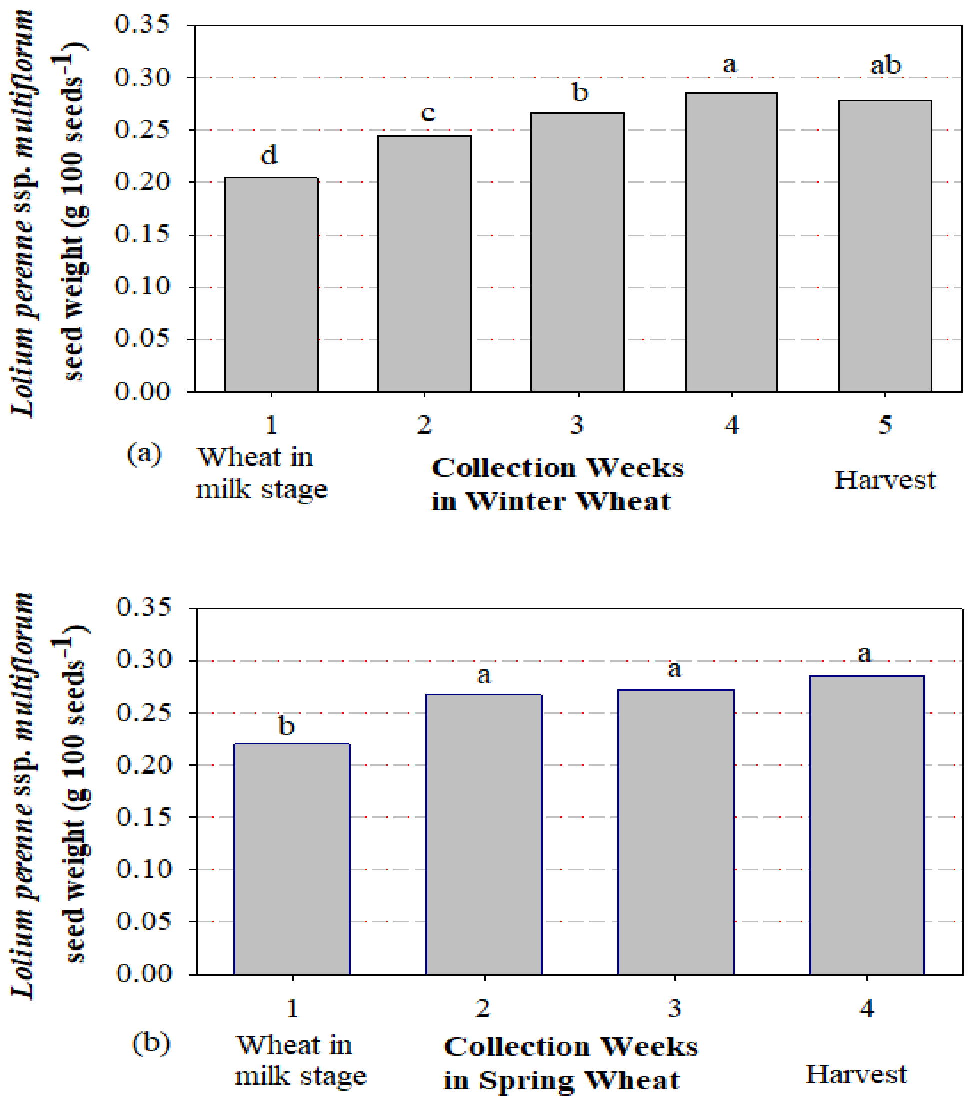

Figure 7. *Lolium perenne* ssp. *multiflorum* seed weight from seed fill to wheat harvest in 2017 and 2018 winter wheat, combined (a) and 2020 spring wheat (b). Bars on each graph represent least squares means (LSMEANS) of seed weight at each collection week and bars with the same letter are not different (α=0.05). Differences between means were determined using pair-wise comparisons of LSMEANS with the GLIMMIX procedure of SAS.

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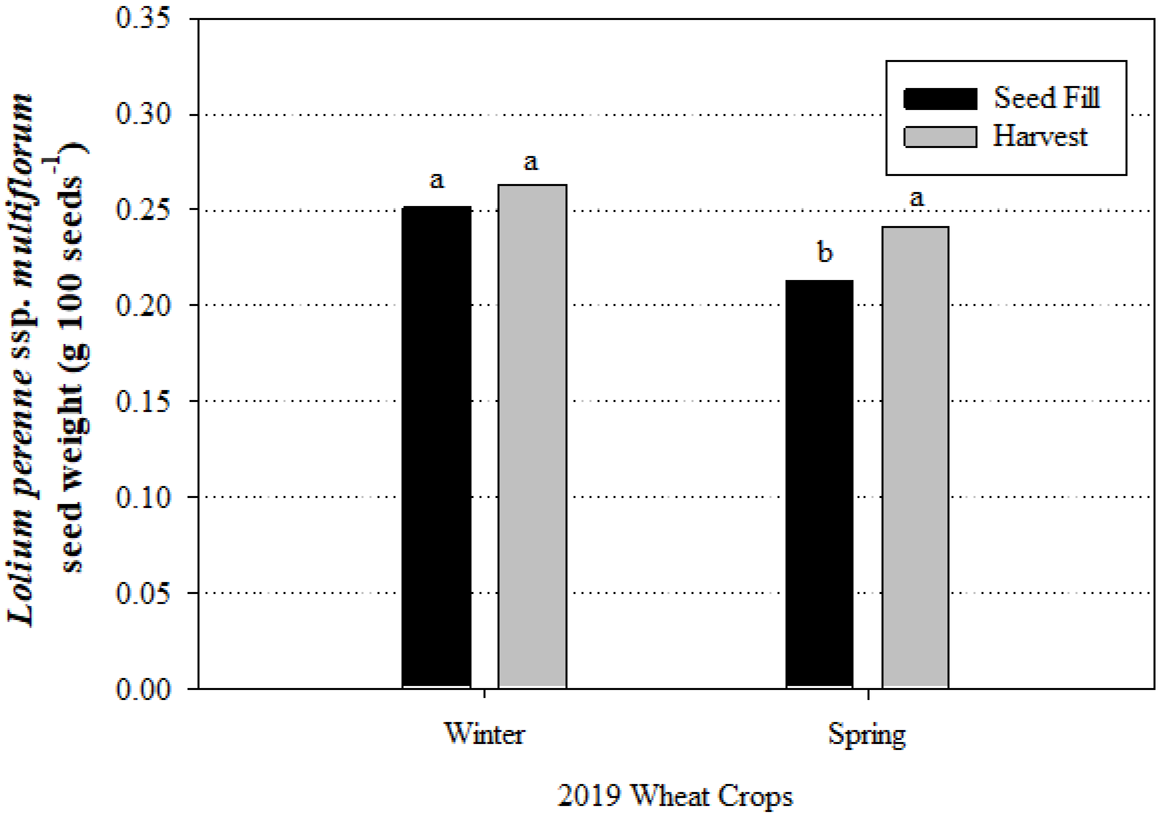

Figure 8. *Lolium perenne* ssp. *multiflorum* seed weight in 2019 winter wheat and spring wheat as affected by collection weeks from seed fill and at crop harvest. Bars with the same letter within each crop are not different α=0.05. Differences between means were determined using pair-wise comparisons of least squares means with the GLIMMIX procedure of SAS.

Figure 9. *Lolium perenne* ssp. *multiflorum* spikelets with florets and glumes.

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