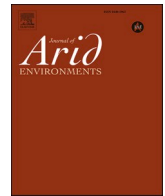


Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

## Journal of Arid Environments

journal homepage: [www.elsevier.com/locate/jaridenv](http://www.elsevier.com/locate/jaridenv)

# An assessment of seed head and flag leaf contributions to reproductive effort in sagebrush steppe bunchgrasses<sup>☆</sup>

Erik P. Hamerlynck<sup>\*</sup>, Rory C. O'Connor

US Department of Agriculture, Agricultural Research Service, Eastern Oregon Agricultural Research Center, Burns, OR, USA

## ARTICLE INFO

### Keywords:

*Agropyron cristatum*  
*Elymus elymoides*  
 Maternal investment  
 Rangeland restoration  
 Seed mass  
 Specific seed mass

## ABSTRACT

North American sagebrush-steppe bunchgrasses primarily establish from seed; however, the importance of parental plant carbon from flag leaves or within the seed head itself to reproduction in perennial bunchgrasses is unknown. To address this, we experimentally removed flag leaves and shaded seed heads to assess their importance to reproduction in the exotic bunchgrass crested wheatgrass (*Agropyron cristatum*), which has high seed head photosynthetic capacity and readily establishes from seed, and the native grass, squirreltail (*Elymus elymoides*), which has lower seed head photosynthetic capacity and does not establish as readily from seed. We hypothesized that inflorescence shading would have a greater negative impact on crested wheatgrass reproduction than in squirreltail. In crested wheatgrass, shading did not affect total propagule production but did reduce both total filled seeds and filled seed specific mass (dry mass per unit area). Flag leaf removal stimulated seed filling and increased seed specific mass, especially in unshaded seed heads, suggesting flag leaves are competitive carbon sinks in crested wheatgrass. In contrast, flag leaf removal and shading in squirreltail resulted in similar reductions in total propagules, fewer filled seed produced and lower specific seed mass, indicating similar contributions to reproductive effort by both structures. These results indicate seed head photosynthetic activity is an adaptive reproductive feature in both grasses, but the contrasting effects of flag leaf removal may reflect long-term adaptive responses to grazing pressures that differed in crested wheatgrass's native range compared to those in North American sagebrush steppe.

## 1. Introduction

Population dynamics of arid and semi-arid perennial bunchgrasses depends largely on sexual reproduction and production of viable seed cohorts (Liston et al. 2003; Hamerlynck and Davies 2019). Understanding the mechanisms contributing to bunchgrass seed production and subsequent seedling establishment success is critical to informing efforts to restore sagebrush-steppe rangelands of the Intermountain Western US. In addition to impacts of historical overgrazing, the integrity of millions of hectares of these ecologically and economically important rangelands has been degraded by competition from invasive annual grasses and associated accelerated fire cycles that are likely to be exacerbated by ongoing climate change (Chambers et al. 2014). Establishing self-perpetuating perennial bunchgrass populations is the most economical and effective method to restore degraded sagebrush steppe

(Morris et al. 2011), but is plagued by very low success rates, especially for native grasses (Chambers et al. 2014; Clements et al. 2017). As such, there has been considerable research assessing seedling trait and ecophysiological characteristics related to post-emergent seedling performance and establishment success (Jones et al. 2010; Rowe and Leger 2011; Hamerlynck et al. 2016; Bell et al. 2019; Mukherjee et al. 2019). Additional attention has been given to modifying seeding rate strategies and developing seed coating technologies aimed improving survival through emergence, considered a strong demographic bottleneck to establishing lasting sagebrush steppe bunchgrass populations (James et al. 2011; Madsen et al. 2014; Schantz et al. 2016; Hardegreer et al. 2020).

In addition to the functional characteristics of the seedling itself, parental plant performance and investment, including photosynthetic activity by reproductive structures, can also affect seedling performance

<sup>☆</sup> EOARC is jointly operated by the USDA-ARS and the Oregon State University Agricultural Experiment Station. USDA is an equal opportunity provider and employer. Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA-ARS or the authors and does not imply approval to the exclusion of other products.

<sup>\*</sup> Corresponding author. USDA-ARS, Eastern Oregon Agricultural Research Center 67826-A Hwy. 205, Burns, OR, 97720, USA.

E-mail addresses: [Erik.hamerlynck@usda.gov](mailto:Erik.hamerlynck@usda.gov), [Erik.hamerlynck@oregonstate.edu](mailto:Erik.hamerlynck@oregonstate.edu) (E.P. Hamerlynck).

<https://doi.org/10.1016/j.jaridenv.2021.104442>

Received 9 September 2020; Received in revised form 4 January 2021; Accepted 7 January 2021

Available online 14 January 2021

0140-1963/Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

(Bazzaz et al. 1979; Roach and Wulff 1987; Huxman et al. 1998; Raven and Griffiths 2015). Parental plant investment has received little study in rangeland perennial bunchgrasses, and its effects vary considerably in the success or failure of locally adapted native grasses in response to competitive regimes imposed by non-native grasses (Espeland and Hammond 2013). Indeed, some studies suggest that bunchgrass seed production and quality are independent of maternal plant performance (Drenovsky et al. 2016). However, these findings are based solely in terms of maternal plant biomass and did not assess performance of the main structures associated with maternal investment in grasses: the photosynthetic contributions by the flag leaf and within the seed head itself.

While well studied in cereal grain crops (Austin et al. 1982; Ziegler-Jöns 1989; Wechsung et al. 2001; Abbad et al. 2004; Tambussi et al. 2007; Sanchez-Bragado et al. 2016) and commercial turfgrasses (Ong et al. 1978; Warringa and Marinissen 1997; Warringa et al. 1998), seed head and flag leaf photosynthetic contributions to reproductive effort are largely unknown in semi-arid perennial bunchgrasses. Recently, Hamerlync et al. (2019) conjectured that variation in seed head and flag leaf photosynthesis may contribute to the observed variation in maternal effects on bunchgrass seedling performance and growth habit (Rowe and Leger 2011; Espeland and Hammond 2013). Hamerlync et al. (2019) found that seed heads of the exotic crested wheatgrass had photosynthetic capacity and carbon fixation efficiency similar levels to flag leaves. Even though seed head photosynthetic capacity was lower in native grasses, these were comparable to those of high-yielding wheat cultivars (Tambussi et al. 2007), while flag leaf photosynthetic performance of native grasses was similar to the exotic grass. Hamerlync et al. (2019) suggested that crested wheatgrass' higher seed head photosynthetic capacity and efficiency may contribute to its higher overall investment to reproductive productivity. This investment may possibly underlie the ability of crested wheatgrass to consistently produce viable seed cohorts, even under drought and extensive strong herbicide application (Wilson and Pärtel 2003; Hamerlync and Davies 2019).

Though Hamerlync et al. (2019) did show crested wheatgrass produced seed heads with 2.5 to 4x greater specific mass (dry mass per unit area) compared to native grasses, they did not determine reproductive effort apportioned by seed head and flag leaf processes in the species studied. While the effects of soil texture and moisture, nitrogen fertilization, burning and grazing to bunchgrass seed productivity and viability have been studied (Coffin and Lauenroth 1992; Dyer 2002; Drenovsky et al. 2016), the importance of variation in seed head and flag leaf contributions remain unknown. To address this, we implemented a factorial field study, experimentally clipping flag leaves and shading post-anthesis seed heads of the exotic grass, crested wheatgrass (*Agropyron cristatum*), and native squirreltail (*Elymus elymoides*). These perennial bunchgrasses were chosen because they differ in (i) seed head photosynthetic capacity and seed head specific mass (Hamerlync et al. 2019; Hamerlync and Ziegenhagen 2020) and (ii) ability to establish and spread from seed (Hamerlync and Davies 2019), both of which are markedly higher in crested wheatgrass.

We measured the following aspects of reproductive effort: 1) seed head mass and specific mass ( $\text{mg cm}^{-2}$ ), and total propagule production, which are indicative of overall total allocation towards reproduction. We then determined aspects related to reproductive potential by 2) quantifying propagule production in terms of filled and unfilled seeds, and 3) propagule quality, in terms of individual seed mass and specific mass for both filled and unfilled seeds. We included filled and unfilled seeds as they represent realized and unrealized potentials of total reproductive effort. Also, it may be that flag leaf and seed head inputs play different roles in determining the number and quality of any seed produced, regardless of its potential viability. Based on our two selection criteria, we specifically hypothesized that crested wheatgrass reproductive effort would respond more strongly to seed head shading, while that in squirreltail would respond more strongly to flag leaf removal

compared to the exotic grass.

## 2. Materials and methods

Field work was conducted from May 30 – July 20, 2019 at the Northern Great Basin Experimental Range (NGBER; 119°43'W, 43°29'N), located approximately 70 km west of Burns, OR, at an elevation 1402 m ASL. Mean annual temperature at the site is 14.8 C, ranging from average daily maximums of 28.7 C in July to -7.1 C in January. Mean annual precipitation is 278.4 mm, primarily as rain, approximately 71% of this distributed evenly across the cool-season period spanning November–May, with occasional snowfall over the coldest months. Soils at the site are classified as a Vil-Decantel-Ratto complex, consisting of well-drained loamy to sandyloam Aridic Durixerols with an indurated illuvial silica duripan (Bqm) beginning at 30–50 cm (Lentz and Simonson 1986). The plants sampled were selected from adult individuals established in a 14 × 145 m (2030 m<sup>2</sup>) plot used for seedling demography studies from 2006 to 2011. *Elymus elymoides* seed sources were Toe Jam Creek germplasm from North Basin Seed, Yakima, WA (Lot# NBS-CF5-TJC-1). Seed sources for *Agropyron cristatum* were Hycrest (Granite Seed, Lehi, UT AGDE Lot# 33426) and Hycrest II (Bruce Seed Farm, Townsend, MT, Lot# 1480-BBHY9A). As the original studies did not differentiate between the crested wheatgrass cultivars, we could not assess differences in their reproductive attributes. Prior to planting, the plot was tilled, and all grass and shrub matter removed. A perimeter fence has kept the area free of rodents and livestock herbivory since its establishment. All plants sampled in this study originally planted in 2007 and 2008 and were clipped to ca. 2.5 cm from the crown the previous year in a study assessing seed head photosynthetic responses to defoliation (Hamerlync and Ziegenhagen 2020).

Within each species, five plants were assigned randomly for flag leaf removal (clipped and unclipped controls) and seed head shading treatments (bagged and unbagged controls), to achieve a full 2 × 2 factorial flag leaf by seed head shading treatment combination (total n = 40 plants). All flag leaves were removed twice weekly from reproductive culms when swelling at base of terminal node was noticed prior to seed head emergence. Seed head shading was achieved by enclosing post-anthesis seed heads with sheaths made from reflective mylar, with five reproductive stems per plant shaded. Due to differences in seasonal phenology, squirreltail seed heads were bagged on June 24 and crested wheatgrass seed heads on July 7. For both species, shade bags were in place for at least 14 days, with squirreltail seed heads harvested before seed head disarticulation commenced on control plants.

We first scanned intact seed heads, if possible, then separated each seed head into filled and unfilled seeds and rachis portions. Each portion was scanned individually at 800 DPI resolution on an Epson 10000 Expression flatbed scanner calibrated to determine total object length and diameter to calculate cylindrical surface area using WinRhizo v2.0 software (Regent Instruments, Ste. Foy, Quebec, Canada). The total number of filled and unfilled empty seeds were manually counted. Each portion was dried at 48 c for a minimum of 7 days, and then weighed to 0.00001 g on a Mettler AT20 microbalance.

From these data, we estimated intact seed head mass (g), specific mass ( $\text{mg cm}^{-2}$  total seed head area), total propagule production (filled seed + empty developed seed + empty undeveloped seed), total seed mass (filled and empty), and average individual filled and empty seed mass (total mg/total seeds) and individual seed specific mass ( $\text{mg cm}^{-2}$ ; total fraction mass/total fraction area). For our estimation of total propagule production, empty seeds were separated into developed and undeveloped (likely aborted) categories. Since the total number and size of the latter were so small, these were included with unfilled developed seeds for estimations of unfilled seed mass and specific mass.

Two-way analysis of variance (two-way ANOVA; Statistix v8.0; Analytical Software, Tallahassee, FL) was used to compare the factorial combinations of flag leaf removal and seed head shading treatments and their interaction effect within each species. As we were interested in

quantitative rather than proportional metrics of reproductive effort, we focused our analyses on differences in the patterns of significance in the obtained two-way ANOVA as indicative of species-specific responses. Where appropriate, we used one-way ANOVA (Statistix v8.0; Analytical Software, Tallahassee, FL) to assess overall species differences pooled across all treatment combinations. Due to stem breakage and the occasional loss of sheaths, we used each flowering stem as a unit of replication. Post-hoc means tests made via  $\alpha$ -corrected LSD, with all differences considered significant at an associated p-value of 0.05.

### 3. Results

#### 3.1. Whole seed head responses

Flag leaf removal did not affect crested wheatgrass seed head mass, while shading reduced seed head mass by 21% compared to unshaded controls (Table 1;  $p < 0.001$ , 1,91 df; Fig. 1a) with no significant flag leaf by shading treatment interaction (Table 1). Shade-induced reduction of seed head mass was more pronounced in unclipped control culms than in clipped culms (Fig. 1a). For squirreltail, there was a significant flag leaf-by-shading treatment interaction (Table 1,  $p = 0.023$ , 1,92 df). In unclipped squirreltail culms, shading reduced seed head mass 29% compared to unshaded controls, while culms without flag leaves showed no difference between shading treatments (Fig. 1a). Flag leaf removal increased crested wheatgrass seed head specific mass by 7% compared to unclipped controls (Table 1;  $p = 0.012$ , 1,91 df) while shading significantly reduced specific mass by 12% ( $p < 0.001$ , 1,91 df), with no interaction effect (Table 1; Fig. 1b). Squirreltail seed head specific mass was unaffected by flag leaf removal but reduced 23% in shaded seed heads (Table 1;  $p < 0.001$ , 1,92 df; Fig. 1b) with no interaction effect (Table 1).

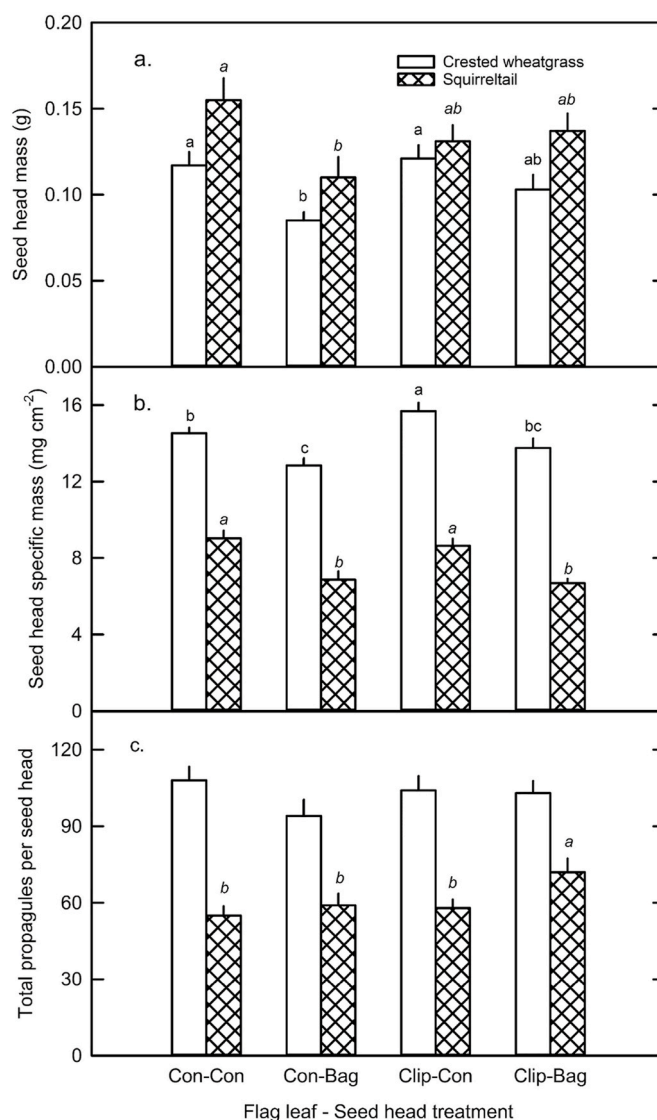
Pooled across all treatment combinations, total propagules produced per seed head differed between the species (One-way ANOVA  $F_{1,48} = 238.7$ ;  $p < 0.001$ ), with crested wheatgrass producing ca. 68% more propagules per seed head ( $102.4 \pm 2.77$  SE) than squirreltail ( $60.8 \pm 2.18$  SE). Total crested wheatgrass propagule production did not significantly respond to flag leaf removal, seed head shading, with no interaction effect (Table 1; Fig. 1c). In contrast, total propagules produced by squirreltail decreased 13% with flag removal ( $p = 0.046$ , 1,88 df; Fig. 1c), while shading increased overall propagule production by 16% ( $p = 0.037$ , 1,88 df; Fig. 1c), with no significant interaction (Table 1). Both significant effects were driven by markedly higher production in clipped/shaded seed heads compared to more moderate gains from their respective controls (Fig. 1c).

Overall, crested wheatgrass produced significantly fewer filled seeds per seed head ( $7.3 \pm 1.16$  SE) than squirreltail ( $27.0 \pm 1.95$  SE) pooled across all treatments (One-way ANOVA  $F_{1,48} = 131.28$ ;  $p < 0.001$ ). On

**Table 1**

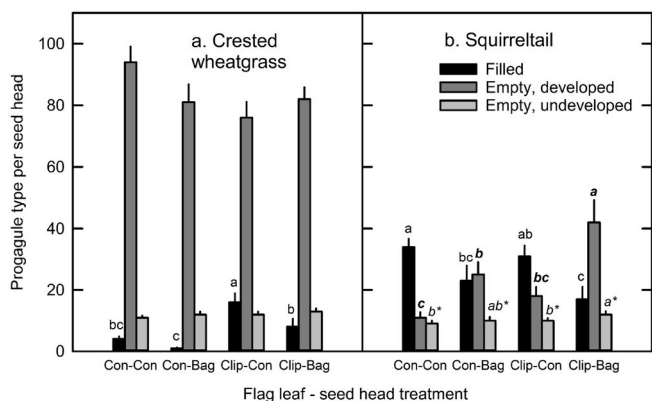
Two-way analysis of variance (ANOVA) F-test results comparing effects of flag leaf removal (Flag) and seed head shading (Shade) on seed head mass, specific mass, and per seed head propagule production in crested wheatgrass and squirreltail. Bold F-test results are significant at  $p < 0.05$ , degrees freedom and p-values are in 3.1.

Seed head attribute	Crested wheatgrass			Squirreltail		
	Flag	Shade	Flag x shade	Flag	Shade	Flag x shade
Mass (g)	2.31 <sup>ns</sup>	<b>11.74</b>	1.01 <sup>ns</sup>	0.02 <sup>ns</sup>	3.16 <sup>ns</sup>	<b>5.35</b>
Specific mass (mg cm <sup>-2</sup> )	<b>6.59</b>	<b>20.04</b>	0.08 <sup>ns</sup>	0.59 <sup>ns</sup>	<b>30.02</b>	0.09 <sup>ns</sup>
Total propagules	0.18 <sup>ns</sup>	2.07 <sup>ns</sup>	1.40 <sup>ns</sup>	<b>4.09</b>	<b>4.47</b>	1.37 <sup>ns</sup>
Filled seed	<b>24.73</b>	<b>7.77</b>	1.82 <sup>ns</sup>	1.52 <sup>ns</sup>	<b>11.39</b>	0.13 <sup>ns</sup>
Empty developed seed	2.78 <sup>ns</sup>	0.56 <sup>ns</sup>	3.48 <sup>ns</sup>	<b>7.31</b>	<b>19.61</b>	1.63 <sup>ns</sup>
Empty undeveloped seed	0.42 <sup>ns</sup>	2.53 <sup>ns</sup>	0.01 <sup>ns</sup>	1.69 <sup>ns</sup>	<b>4.34</b>	0.49 <sup>ns</sup>



**Fig. 1.** Seed head (a) mass, (b) specific mass, and (c) total propagule production of crested wheatgrass (open bars) and squirreltail (cross-hatch bars) flowering culms with intact or removed flag leaves (Con, Clip) and with unshaded or shaded seed heads (Con, Bag). Each bar is the mean of 22–25 observations, error bars indicate  $\pm$  one S.E., letters differ significantly within a species (LSD two-way ANOVA).

an individual species basis, flag leaf removal increased crested wheatgrass filled seed production 5.5-fold from that of unclipped controls ( $p < 0.001$ , 1, 96 df; Fig. 2a), while shading reduced filled seed production by 56% ( $p = 0.006$ , 1,96 df; Fig. 2a), with no significant interaction (Table 1). In contrast, clipping and shading did not affect empty seed production, either for empty fully developed or undeveloped seeds (Table 1; Fig. 2a). In squirreltail, flag leaf removal did not affect filled seed or undeveloped empty seed production (Table 1). Shading significantly reduced filled seed production 38% ( $p = 0.001$ , 1,88 df) and increased undeveloped empty seed production by 24% ( $p = 0.040$ , 1,88 df; Fig. 2b). Removing flag leaves increased the number of developed empty squirreltail seeds by 64% ( $p = 0.008$ , 1,88 df), and shading by 132% ( $p < 0.001$ , 1,88 df), mainly due to large numbers in the clipped/shaded treatment (Fig. 2b). All squirreltail propagule types no treatment interaction effects (Table 1).



**Fig. 2.** Total seeds per seed head filled (black), empty developed (dark gray) or empty undeveloped (light gray) in (a) crested wheatgrass and (b) squirreltail from flowering culms with intact or removed flag leaves (Con, Clip) and unshaded or shaded seed heads (Con, Bag). Each bar is the mean of 22–25 observations, error bars indicate ± one SE, letters differ significantly within a species (LSD, two-way ANOVA).

3.2. Individual seed responses

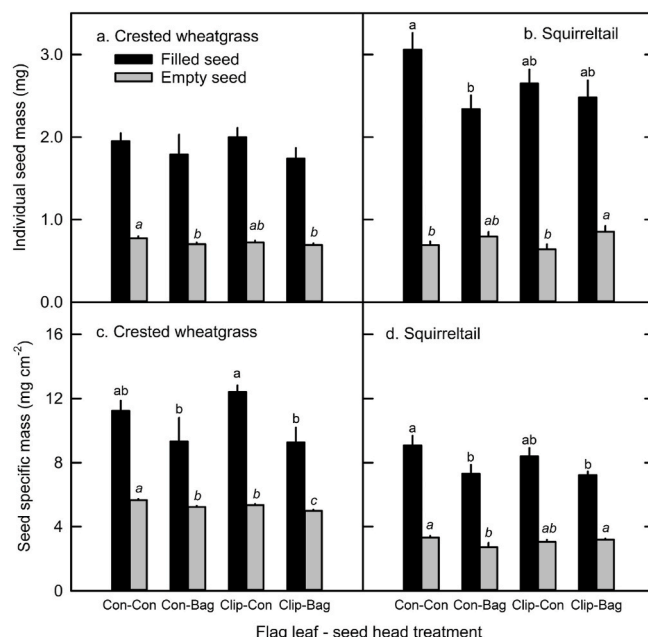
Squirreltail produced individual filled seeds of greater mass (2.68 mg ± 0.099 SE) than those of crested wheatgrass (1.89 mg ± 0.066 SE; One-way ANOVA  $F_{1,48} = 31.08$ ;  $p < 0.001$ ), while individual empty seed mass did not differ between the species. The mass of individual filled crested wheatgrass seeds did not respond to any of the experimental treatments (Table 2; Fig. 3a), while empty seeds mass reduced 7% in shading treatments from unshaded controls ( $p = 0.0198$ , 1,96 df; Fig. 3a). Both filled and empty squirreltail seed masses differed between shading treatments ( $p = 0.0208$ , 1,76 df and  $p = 0.0080$ , 1.88 df, for filled and empty seed masses, respectively), with no flag leaf removal or clipping-by-shading treatment interaction effects (Table 2). Shading reduced filled squirreltail individual seed mass 15% compared to those in unshaded controls, while empty seed mass increased by 25% in shaded seed heads (Fig. 3b).

Crested wheatgrass and squirreltail specific seed mass differed significantly, both for filled (One-way ANOVA  $F_{1,48} = 24.16$ ;  $p < 0.001$ ) and empty seeds (One-way ANOVA  $F_{1,48} = 507.7$ ;  $p < 0.001$ ). Crested wheatgrass filled (10.90 mg cm<sup>-2</sup> ± 0.406 SE) and empty seeds (5.31 mg cm<sup>-2</sup> ± 0.044 SE) had higher specific mass than those of squirreltail (8.19 mg cm<sup>-2</sup> ± 0.284 SE and 3.09 mg cm<sup>-2</sup> ± 0.077 SE for filled and empty seeds, respectively). Flag leaf clipping did not affect filled seed specific mass in either species (Table 2), while shading reduced filled seed specific mass 21% in crested wheatgrass ( $p = 0.0022$ , 1,66 df; Figs. 3c) and 17% in squirreltail ( $p = 0.0121$ , 1,76 df; Fig. 3d), with no significant treatment interaction effect in either species (Table 2). Specific mass of empty crested wheatgrass seeds reduced 5% with flag leaf

**Table 2**

Two-way ANOVA F-test results for differences in individual seed mass, and specific mass of filled and empty seeds of flowering stems of crested wheatgrass and squirreltail flowering culms with intact or removed flag leaf (Flag) and shaded or unshaded seed heads (Shade). Bold F-test results significant at  $p < 0.05$ , degrees freedom and probability provided in 3.3.

Seed type and attribute	Crested wheatgrass			Squirreltail		
	Flag	Shade	Flag x shade	Flag	Shade	Flag x shade
Filled mass (mg)	0.01 <sup>ns</sup>	2.22 <sup>ns</sup>	0.12 <sup>ns</sup>	0.48 <sup>ns</sup>	<b>5.02</b>	2.00 <sup>ns</sup>
Filled specific mass (mg cm <sup>-2</sup> )	0.48 <sup>ns</sup>	<b>10.19</b>	0.61 <sup>ns</sup>	0.44 <sup>ns</sup>	<b>6.62</b>	0.27 <sup>ns</sup>
Empty mass (mg)	2.06 <sup>ns</sup>	<b>5.62</b>	0.91 <sup>ns</sup>	0.01 <sup>ns</sup>	<b>7.38</b>	0.89 <sup>ns</sup>
Empty specific mass (mg cm <sup>-2</sup> )	<b>13.54</b>	<b>27.86</b>	0.22 <sup>ns</sup>	0.39 <sup>ns</sup>	2.26 <sup>ns</sup>	<b>5.59</b>



**Fig. 3.** Filled (black bars) and empty (gray bars) individual seed mass of (a) crested wheatgrass and (b) squirreltail and individual seed specific mass of (c) crested wheatgrass and (d) squirreltail produced from flowering culms with intact or removed flag leaves (Con, Clip) and unshaded or shaded seed heads (Con, Bag). Each bar is the mean of 22–25 observations, error bars indicate ± one S.E., letters differ significantly within a species (LSD, two-way ANOVA).

removal and 7% with shading from their respective controls ( $p < 0.001$ , 1,96 df for both treatments, respectively; Fig. 3c) with no treatment interaction (Table 2). While flag leaf clipping and shading did not affect specific mass of empty squirreltail seeds, there was a significant flag leaf by shading treatment interaction ( $p = 0.0203$ , 1,88 df; Table 2). This was due to shading reducing empty seed specific mass 18% in unclipped culms, while shading did not affect empty seed specific mass on culms with flag leaves removed (Fig. 3d).

4. Discussion

Our hypothesis that reproductive effort in the exotic crested wheatgrass would be more strongly affected by seed head shading, while that of the native grass squirreltail would respond more strongly to flag leaf removal was partially supported. For crested wheatgrass, overall reproductive allocation, as indicated by seed head mass and specific mass, was reduced by seed head shading while total reproductive potential, as indicated by total propagule production was not (Fig. 1). For the native grass squirreltail overall reproductive allocation and reproductive potential did not vary across three of the four treatment combinations but was unexpectedly higher in both with flag leaf removal and shading, though seed head specific mass clearly decreased with shading (Figs. 1 and 2). Thus, seed head photosynthetic processes are likely more important than we hypothesized for this grass. As subsequent discussion will show, these distinct responses were underlain by marked but very different effects on seed filling and individual seed quality that principally resulted from seed head shading.

Our results suggest that overall, photosynthetic processes within the seed head itself are very important to reproductive effort in these arid-land grasses. This agrees with studies of the one perennial grass species previously examined (Ong et al. 1978; Warringa and Marinissen 1997; Warringa et al. 1998), which differ markedly from most annual cereal grasses, which tend to rely more strongly of flag leaf activity for overall head yield and degree of grain filling (Austin et al. 1982; Ziegler-Jöns 1989; Tambussi et al. 2007; Sanchez-Bragado et al. 2016). Moreover,

our results suggest the potential for maternal investment is present, as flag leaf and photosynthetic seed heads are maternal tissue. This stands in contrast to Dervosky et al. (2016), likely because the total amount of biomass in flag leaves and seed heads is dwarfed by total biomass, the metric these researchers used to relate parental plant performance to seed quality and seedling performance.

As noted above, production of squirreltail propagules were greatest in clipped and shaded stems (Fig. 1). It may be any reduction in carbon from within the seed head as well as the flag leaf may be large enough to strongly limit seed metabolic activity, thereby reducing seed-to-seed competition. In perennial ryegrass, experimental removal of seeds showed there is strong seed-seed competition for parentally allocated carbon (Warringa et al. 1998). In clipped/shaded squirreltail, there was a marked increase in unfilled and undeveloped seeds concurrent with a sharp decline in filled seeds compared to the other treatment combinations. Thus, it may be that curtailing carbon available from the flag leaf and seed head reduced competitive displacement that reduced the number of seeds in the other treatments, resulting in higher propagule production. However, this may not be the case with crested wheatgrass, where total propagule number didn't differ between treatments. This was mainly because the majority of seeds were fully developed but empty, and the number of these did not differ across treatments. While having a smaller proportion of actively filling seeds may lower seed-seed competition within the seed head, it is unclear if this is adaptive, given the very low number of filled seed – and hence realized reproductive potential – in crested wheatgrass.

It has long been recognized that seed mass is an important trait in plant functional ecology. Seed mass correlates with seedling developmental growth patterns, nutrient and energetic reserves, and the ability to establish across contrasting habitats and geographic ranges (Harper et al. 1970; Mazer 1989; Kidson and Westoby 2000; Henerly and Westoby 2005; Moles et al. 2005, 2007; Guo et al. 2010). Seed mass has genetic and maternal plant investment components (Harper et al. 1970; Huxman et al. 1998; Castro 1999; Guo et al. 2010; Mukherjee et al. 2019). Our findings of lower seed number and higher seed mass in squirreltail compared to crested wheatgrass agree with other studies showing similar negative relationships between seed number and individual seed mass (Mazer 1989; Guo et al. 2010). Squirreltail is a relatively short-lived, small-statured, early-successional species with greater proportional biomass allocation to reproduction compared to later-successional native sagebrush-steppe perennial bunchgrasses (Drenovsky et al. 2016). In a geo-referenced co-planting study, Hamerlync and Davies (2019) showed that 13 years after co-planting, most crested wheatgrass and squirreltail plants occurred outside their original plantings, but that the longer-lived crested wheatgrass had marked population increase while squirreltail numbers declined precipitously over the same time period. This is somewhat at odds with the greater number of heavier filled seeds of squirreltail compared to crested wheatgrass we observed in this study. Perhaps seed mass may not provide the full resolution needed to assess seed quality.

Our results suggest specific seed mass may be a better indicator of seed quality than seed mass. This can be seen in the markedly lower variation in crested wheatgrass individual seed mass (especially in filled seeds), that still result in higher seed specific mass across all treatments compared to squirreltail. The overall higher seed specific mass in both filled and empty seeds suggests that crested wheatgrass invests greater energetic resources in reproductive propagules. The attainment of this, as the reduction of empty and filled seed specific mass by seed head shading, is driven by the greater investment in photosynthetic capacity within the seed head noted in our previous study (Hamerlync et al. 2019).

Investment in seed tissue density, not just total seed number and seed mass, may give crested the demographic edge it enjoys. However, this likely comes at a cost to realized reproductive potential, as crested wheatgrass filled far fewer propagules. Also, unlike squirreltail which produces long, thin awns that aid in seed dispersal (USDA-NRCS 2006),

crested wheatgrass has very short awns. Dispersal mechanisms and allocation to dispersal structures can impose a trade-off with seed mass (Greene and Johnson 1993; Moles et al. 2005). Though not the major portion of squirreltail total seed area (data not shown), and likely an even smaller portion of seed mass, the presence of awns likely contributed to filled squirreltail seeds having lower specific mass, while specific mass of filled crested wheatgrass seeds is more likely closer to the actual density of the energetic reserves of the seed.

Squirreltail produced similar total numbers of filled seeds with similar seed specific mass between unclipped/shaded and clipped/unshaded culms. These findings suggest both flag leaves and seed head photosynthesis provide energetic inputs to filled squirreltail seeds, with the edge going towards seed head sources, given that clipped/unshaded seed number and specific mass was closer to those attained in unmanipulated control culms. This was decidedly not the case in crested wheatgrass, where flag leaf removal led to more filled seeds, especially when paired with unshaded seed heads, and in filled seeds with highest specific mass. This unexpected result suggests that, unlike in squirreltail, crested wheatgrass flag leaves compete with the seed head for resources. In some cereal crops, flag leaves can act as competitive sinks for nutrients (Chapin and Wardlaw, 1988; Aranjuelo et al. 2013) and the relative degree of sink/source activity in seed heads and flag leaves can vary with soil-water availability and genotype, affecting overall biomass and grain yields (Blum et al. 1988; Abbad et al. 2004). In such cases, human selection for high yields in annual grasses has driven development of seed head and flag leaf photosynthetic capacity and may also underlie the nature of source/sink relationships between these structures, so extrapolating to perennial grasses growing in more natural and fluctuating conditions should be approached with caution.

Long-term natural selection for grazing tolerance in crested wheatgrass may why flag leaf removal stimulated seed filling and improved seed quality via greater filled seed specific mass. Crested wheatgrass is native to steppe/grassland systems that have long supported a diverse array of extensive and intensive grazing, often under considerable human management (Rogler and Lorenz 1983). Compared to native bunchgrasses, crested wheatgrass seed heads have maximum photosynthetic capacity and carbon-fixation efficiencies that equal or exceed those of flag leaves (Hamerlync et al. 2019). Such extensive allocation to photosynthetic capacity of a reproductive structure very likely increased the potential for these to compete with vegetative structures over the course of seasonal development. If this is the case, seed head photosynthesis would be of more importance to crested wheatgrass reproductive effort, as we hypothesized, mainly because the flag leaf may never act as a major carbon source for grain filling, something we had not considered in our experimental design. If so, it may be that crested wheatgrass flag leaves serve a different role in reproduction, such as shielding the pre-emergent seed head from excessive light levels or providing support against mechanistic stresses such as wind or heavy rain.

Greater allocation to seed head photosynthetic capacity would likely make crested wheatgrass reproduction not as susceptible to foliar biomass loss to herbivory, as our results indicate it would be able to compensate by making more seeds of higher quality. These results fit into a growing body of evidence that the success of this grass in North American arid and semi-arid rangelands depends on its ability to optimize plant carbon uptake and allocation processes in response to defoliation at all its demographic stages (Meays et al. 2000; Hamerlync et al. 2016; Hamerlync and Ziegenhagen 2020), and such that it can consistently produce viable seed cohorts to overcome demographic bottlenecks to seedling establishment (James et al. 2011).

Our results advance Hamerlync et al.'s (2019) conjecture that reproductive photosynthetic activity may contribute to variation in maternal effects observed in native grasses that have rapidly evolved in response to competition with invasive annual grasses (Rowe and Leger 2011; Espeland and Hammond 2013). However, given the huge range of current and future climate conditions across the US Great Basin

sagebrush-steppe (Chambers et al. 2014; Hardegee et al. 2020), the relative contributions of flag leaf and seed head photosynthesis to seed number and quality likely vary considerably with interannual seasonal water-availability, temperature and herbivory. Until rigorous experimental approaches are implemented to address these environmental and biotic features, how much so remains unclear.

### Author contribution

Erik Hamerlync: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft preparation, Rory O'Connor: Conceptualization, Writing – original draft preparation

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### References

- Abbad, H., El Jaafari, S., Bort, J., Araus, J.L., 2004. Comparison of flag leaf and ear photosynthesis and biomass and grain yield of durum wheat under various water conditions and genotypes. *Agronomie* 24, 19–28.
- Aranjuelo, I., Cabrera-Bosquet, L., Araus, J.L., Nogués, S., 2013. Carbon and nitrogen partitioning during the post-anthesis period is conditioned by N fertilization and sink strength in three cereals. *Plant Biol.* 15, 135–143.
- Austin, R.B., Morgan, C.L., Ford, M.A., Bhagwat, S.G., 1982. Flag leaf photosynthesis of *Triticum aestivum* and related diploid and tetraploid species. *Ann. Bot.* 49, 177–189.
- Bazzaz, F.A., Carlson, R.W., Harper, J.L., 1979. Contribution to reproductive effort by photosynthesis of flowers and fruits. *Nature* 279, 554–555.
- Bell, B.P., Jones, T.A., Monaco, T.A., 2019. Productivity and morphologic traits of thickspike wheatgrass, Snake River wheatgrass, and their interspecific hybrids. *Rangel. Ecol. Manag.* 72, 73–81.
- Blum, A., Mayer, J., Golan, G., 1988. The effect of grain number per ear (sink size) on source activity and its water-relations in wheat. *J. Exp. Bot.* 39, 106–114.
- Castro, J., 1999. Seed mass versus seedling performance in Scots pine: a maternally dependent trait. *New Phytol.* 144, 153–161.
- Chambers, J.C., Bradley, B.A., Brown, C.S., D'Antonio, C.D., Germino, M.J., Grace, J.B., Hardegee, S.P., Miller, R.F., Pyke, D.A., 2014. Resilience to stress and disturbance, and resistance to *Bromus tectorum* L. invasion in cold desert shrublands of Western North America. *Ecosystems* 17, 360–375.
- Chapin, F.S., Wardlaw, I.F., 1988. Effect of phosphorus deficiency on source-sink interactions between the flag leaf and developing grain in barley. *J. Exp. Bot.* 39, 165–177.
- Clements, C.D., Harmon, D.N., Blank, R.L., Weltz, M., 2017. Improving seeding success in cheatgrass-infested rangelands in Northern Nevada. *Rangelands* 39, 174–181.
- Coffin, D.P., Lauenroth, W.K., 1992. Spatial variability in seed production of the perennial bunchgrass, *Bouteloua gracilis*. *Am. J. Bot.* 79, 347–353.
- Drenovsky, R.E., Thornhill, M.L., Knestrick, M.A., Dlugos, D.M., Svejcar, T.J., James, J.J., 2016. Seed production and seedling fitness are uncoupled from maternal plant productivity in three aridland bunchgrasses. *Rangel. Ecol. Manag.* 69, 161–168.
- Dyer, A.R., 2002. Burning and grazing management in a California grassland: effect on bunchgrass seed viability. *Restor. Ecol.* 10, 107–111.
- Espeland, E.K., Hammond, D., 2013. Maternal effects on growth and competitive ability in a commonly used restoration species. *Native Plants J.* 14, 231–242.
- Greene, D.F., Johnson, E.A., 1993. Seed mass and dispersal capacity in wind-dispersed diaspores. *Oikos* 67, 69–74.
- Guo, H., Mazer, S.J., Du, G., 2010. Geographic variation in seed mass within and among nine species of *Pedicularis* (Orobanchaceae): effects of elevation, plant size, and seed number per fruit. *J. Ecol.* 98, 1232–1242.
- Hamerlync, E.P., Smith, B.S., Sheley, R.L., Svejcar, T.J., 2016. Compensatory photosynthesis, water-use efficiency and biomass allocation of defoliated exotic and native bunchgrass seedlings. *Rangel. Ecol. Manag.* 69, 206–214.
- Hamerlync, E.P., Davies, K.W., 2019. Changes in abundance of eight sagebrush-steppe bunchgrass species 13 yr. after co-planting. *Rangel. Ecol. Manag.* 72, 23–27.
- Hamerlync, E.P., Denton, E.M., Davies, K.W., Boyd, C.S., 2019. Photosynthetic regulation in seed heads and flag leaves of sagebrush-steppe bunchgrasses. *Conserv. Physiol.* 7, coz112.
- Hamerlync, E.P., Ziegenhagen, L.L., 2020. Seed head photosynthetic light responses in clipped and unclipped sagebrush steppe bunchgrasses. *J. Arid Environ.* 172, 104031.
- Hardegee, S.P., Sheley, R.L., James, J.J., Reeves, P.A., Richards, C.M., Walters, C.T., Boyd, C.S., Moffet, C.A., Flerchinger, G.N., 2020. Germination syndromes and their relevance to rangeland seeding strategies in the Intermountain Western United States. *Rangel. Ecol. Manag.* 73, 334–341.
- Harper, J.J., Lovell, P.H., Moore, K.G., 1970. The shapes and sizes of seeds. *Annu. Rev. Ecol. Systemat.* 1, 327–356.
- Henery, M.L., Westoby, M., 2005. Seed mass and seed nutrient content as predictors of seed output variation between species. *Oikos* 92, 479–490.
- Huxman, T.E., Hamerlync, E.P., Jordan, D.N., Salsman, K.J., Smith, S.D., 1998. The effects of parental CO<sub>2</sub> environment on seed quality and subsequent seedling performance of *Bromus rubens*. *Oecologia* 114, 202–208.
- James, J.J., Svejcar, T.J., Rinella, M.J., 2011. Demographic processes limiting seedling recruitment in arid grassland restoration. *J. Appl. Ecol.* 48, 961–969.
- Jones, T.A., Monaco, T.A., James, J.J., 2010. Launching the counterattack: interdisciplinary deployment of native-plant functional traits for repair of rangelands dominated by invasive annual grasses. *Rangelands* 32, 38–42.
- Kidson, R., Westoby, M., 2000. Seed mass and seedling dimensions in relation to seedling establishment. *Oecologia* 125, 11–17.
- Lentz, D.R., Simonson, G.H., 1986. A Detailed Soils Inventory and Associated Vegetation of Squaw Butte Experiment Station. Special Report 760. Agric. Exp. Sta., Oregon State Univ., Corvallis, OR.
- Liston, A., Wilson, B.L., Robinson, W.A., Doescher, P.S., Harris, N.R., Svejcar, T., 2003. The relative importance of sexual reproduction versus clonal spread in an aridland bunchgrass. *Oecologia* 137, 216–225.
- Madsen, M.D., Davies, K.W., Boyd, C.S., Kerby, J.D., Svejcar, T.J., 2014. Emerging seed enhancement technologies for overcoming barriers to restoration. *Restor. Ecol.* 24, S77–S84.
- Mazer, S.J., 1989. Ecological, taxonomic, and life history correlates of seed mass among Indiana Dune angiosperms. *Ecology* 59, 153–175.
- Meays, C.L., Laliberte, A.S., Doescher, P.S., 2000. Defoliation response of bluebunch wheatgrass and crested wheatgrass: why we cannot graze these two species in the same manner. *Rangelands* 22, 16–18.
- Moles, A.T., Ackerly, D.D., Webb, C.O., Tweddle, J.C., Dickie, J.B., Pitman, A.J., Westoby, M., 2005. Factors that shape seed mass evolution. *Proc. Nat. Acad. Sci., USA* 102, 10540–10544.
- Moles, A.T., Ackerly, D.D., Tweddle, J.C., Dickie, J.B., Smith, R., Leishman, M.R., Mayfield, M.M., Pitman, A., Wood, J.T., Westoby, M., 2007. Global patterns in seed size. *Global Ecol. Biogeogr.* 16, 109–116.
- Morris, L.R., Monaco, T.A., Call, C.A., Sheley, R.L., 2011. Implementing ecologically based invasive plant management: lessons from a century of demonstration projects, Park Valley, Utah, USA. *Rangelands* 33, 2–9.
- Mukherjee, J.R., Jones, T.A., Monaco, T.A., Adler, P.B., 2019. Relationship between seed mass and young-seedling growth and morphology among nine bluebunch wheatgrass populations. *Rangel. Ecol. Manag.* 72, 283–291.
- Ong, C.K., Colvill, K.E., Marshall, C., 1978. Assimilation of 14CO<sub>2</sub> by the inflorescence of *Poa annua* L. and *Lolium perenne* L. *Ann. Bot.* 42, 855–862.
- Raven, J.A., Griffiths, H., 2015. Photosynthesis in reproductive structures: costs and benefits. *J. Exp. Bot.* 66, 1699–1705.
- Roach, D.A., Wulff, R.D., 1987. Maternal effects in plants. *Annu. Rev. Ecol. Systemat.* 18, 209–235.
- Rogler, G.A., Lorenz, R.J., 1983. Crested wheatgrass – early history in the United States. *J. Range Manag.* 36, 91–93.
- Rowe, C.L.J., Leger, E.A., 2011. Competitive seedlings and inherited traits: a test of rapid evolution of *Elymus multisetus* (big squirreltail) in response to cheatgrass invasion. *Evol. Appl.* 4, 485–498.
- Sanchez-Bragado, R., Molero, G., Reynolds, M.P., Araus, J.L., 2016. Photosynthetic contribution of the ear to grain filling in wheat: a comparison of different methodologies. *J. Exp. Bot.* 67, 2787–2798.
- Schantz, M.C., Sheley, R.L., James, J.J., Hamerlync, E.P., 2016. Role of dispersal timing and frequency in annual grass invaded Great Basin ecosystems: how modifying seeding strategies increases restoration success. *West. N. Am. Nat.* 76, 36–52.
- Tambussi, E.A., Bort, J., Guiamet, J.J., Nogués, S., Araus, J.L., 2007. The photosynthetic role of ears in C<sub>3</sub> cereals: metabolism, water use efficiency and contribution to grain yield. *CRC Crit. Rev. Plant Sci.* 26, 1–16.
- Usda-Nrcs, 2006. Plant guide, bottlebrush squirreltail and big squirreltail, *Elymus elymoides* (raf.) swezey & *Elymus multisetus* M.E. Jones. USDA-NRCS plants database. [https://plants.sc.egov.usda.gov/plantguide/pdf/pg\\_ele15.pdf](https://plants.sc.egov.usda.gov/plantguide/pdf/pg_ele15.pdf). (Accessed 27 July 2020).
- Warringa, J.W., Marinissen, M.J., 1997. Sink-source and sink-sink relationships during reproductive development in *Lolium perenne* L. *Neth. J. Agric. Sci.* 45, 505–520.
- Warringa, J.W., De Visser, R., Kreuzer, A.D.H., 1998. Seed weight in *Lolium perenne* as affected by interactions among seeds within the inflorescence. *Ann. Bot.* 82, 835–841.
- Wechsung, F., Garcia, R.L., Wall, G.W., Kartschall, T., Kimball, B.A., Michaelis, P., Pinter, P.J., Wechsung, G., Grossman-Clarke, S., Lamorte, R.L., Adamsen, F.J., Leavitt, S.W., Thompson, T.L., Matthias, A.D., Borrks, T.J., 2001. Photosynthesis and conductance of spring wheat ears: field response to free-air CO<sub>2</sub> enrichment and limitations in water and nitrogen supply. *Plant Cell Environ.* 23, 917–929.
- Wilson, S.D., Pärtel, M., 2003. Extirpation or coexistence? Management of a persistent introduced grass in a prairie restoration. *Restor. Ecol.* 11, 410–416.
- Ziegler-Jöns, A., 1989. Gas exchange of ears of cereals in response to carbon dioxide and light. *Planta* 178, 84–91.