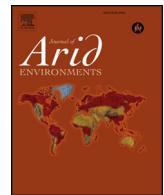




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Seed head photosynthetic light responses in clipped and unclipped sagebrush steppe bunchgrasses

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ABSTRACT

Low seedling establishment limits the long-term success of sagebrush steppe restoration, and the physiological mechanisms underlying this remain unclear. To address this, we measured the photosynthetic light responses and seed head specific length (mg cm^{-1}) of shaded and unshaded seed heads in clipped and unclipped plants to determine if grazing affects reproductive photosynthesis in sagebrush steppe bunchgrasses. We measured responses in an exotic species, *Agropyron cristatum* (crested wheatgrass) that readily establishes from seed, and two native grasses, *Elymus elymoides* (squirretail wild rye) and *Psuedoroegnaria spicata* (bluebunch wheatgrass), which do not. Defoliation did not affect seed head light saturation responses in any of the grasses. *Agropyron cristatum* seed heads attained higher light-saturated photosynthesis and higher photosynthetic light use efficiencies than the native grasses, consistent with its ability to produce viable seed crops. Defoliation and shading reduced post-anthesis seed head specific masses only in *E. elymoides*, suggesting this species reproductive effort relies on carbon fixed by the seed head itself. These findings could help in the selection and development of native plant materials with characteristics similar to the successful exotic grass to improve restoration efforts in degraded sagebrush steppe ecosystems.

1. Introduction

In North America, the stability and resilience of ecologically and economically important sagebrush steppe rangelands are threatened by degradation via the spread of highly competitive exotic annual grasses, which, coupled with ongoing climate change, dramatically alter inter-annual fire severity and frequency in these ecosystems (Davies et al., 2011; Svejcar et al., 2017). Reseeding sagebrush steppe following fire is often the only economically viable way to re-establish sagebrush steppe plant communities (Hardegree et al., 2016; Svejcar et al., 2017). Establishing stable bunchgrass populations capable of maintaining themselves by seed greatly enhances long-term restoration success (Boyd and Davies, 2012; Brooks et al., 2016), as bunchgrass population dynamics are driven by sexual reproduction, rather than by vegetative propagation (Liston et al., 2003; Hamerlynck and Davies, 2019). In addition, grazing is an important ecological, economic and management feature in North American sagebrush steppe rangelands (Svejcar et al., 2017). Defoliation can dramatically alter reproductive effort and success in sagebrush steppe grasses (Anderson and Frank, 2003;

Hamerlynck et al., 2016a). Therefore, understanding the effects of grazing on the mechanisms underlying reproductive effort in bunchgrasses will provide valuable ecological information for improving conservation and restoration efforts in these water-limited rangeland ecosystems.

The exotic perennial *Agropyron cristatum* (L.) Gaertn. (crested wheatgrass) is a valuable species in sagebrush steppe restoration efforts as it readily establishes from seed and can readily outcompete invasive annual grasses (Davies et al., 2010), and has seedlings that can better tolerate defoliation than native grasses (Hamerlynck et al., 2016; Denton et al., 2018). *Agropyron cristatum* can produce viable seed cohorts even when exposed to stringent control (Bakker et al., 2003; Wilson and Pärtel, 2003; Fansler and Mangold, 2011). *Agropyron cristatum* can halt invasive annual grass spread (Davies et al., 2010), and, while still competitive with native grasses, modifies soil conditions to a lesser extent than invasive grasses do (Perkins and Nowak, 2012; Gasch et al., 2016); this may create openings for subsequent native grass establishment, which are typically more difficult to establish from seed (Clements et al., 2017). However, just how *A. cristatum* consistently

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produces more viable seed than do native bunchgrasses is not clear.

Photosynthetic carbon assimilation by reproductive structures can be an important determinant of plant reproductive effort and success (Bazzaz et al., 1979; Raven and Griffiths, 2015), but to date has not been studied in arid or semi-arid perennial grasses. In annual cereal grasses, the flag leaf generally contributes most of the carbon for seed production and filling, though carbon fixed within the seed head itself can exceed flag leaf contributions (Evans and Rawson, 1970; Austin et al., 1982; Ziegler-Jöns, 1989; Wechsung et al., 2001; Tambussi et al., 2007; Rangan et al., 2016; Sanchez-Bragado et al., 2016; Taylor and Long, 2017). In the one perennial grass studied, perennial rye grass (*Lolium perenne* L.), structures within the seed head are the principal source of carbon rather than the flag leaf (Ong et al., 1978; Warringa and Kreuzer, 1996; Warringa and Marinissen, 1997; Warringa et al., 1998). Of these studies, only a few have quantified direct photosynthetic responses of the seed head itself to environmental variability. Wechsung et al. (2001) documented higher photosynthesis of wheat ears under elevated CO₂ and water-limiting conditions compared to levels attained in ambient CO₂, and Ziegler-Jöns (1989) showed increased light penetration following ear opening stimulated whole-ear photosynthesis, even as photosynthetic capacity of glumes and florets declined. Given the importance of photosynthetic processes within the seed head in perennial grasses (Warringa and Kreuzer, 1996; Warringa and Marinissen, 1997; Warringa et al., 1998), *A. cristatum*'s ability to produce viable seed consistently may in part be due to having reproductive photosynthetic characteristics that differ substantially those of native sagebrush steppe bunchgrasses.

Here, we present the results of a field study with the goals of comparing (1) photosynthetic light responses and (2) post-anthesis reproductive productivity in seed heads of clipped and unclipped plants of crested wheatgrass and two native perennial species, *Elymus elymoides* (Raf.) Swezey (squirreltail wild rye) and *Psuedoroegnaria spicata* (Pursh) Å. Löve (bluebunch wheatgrass). Documenting how seed head photosynthesis and reproductive productivity in *A. cristatum* and native grasses vary between defoliated and un-defoliated plants will provide insight on a critical aspect of the functional ecology of these grasses across the grazing mosaics typical of sagebrush steppe ecosystems. To attain our first goal, we generated photosynthetic light saturation response curves to determine seed head light saturated photosynthetic assimilation rate (A_{sat}), dark respiration (R_{dark}), photosynthetic compensation point (I_{comp}) and quantum requirement (Q_{req}). To attain our second goal, we experimentally shaded individual post-anthesis seed heads to reduce seed head photosynthetic carbon inputs to reproductive productivity (Warringa and Marinissen, 1997; Sanchez-Bragado et al., 2016), and compared reproductive biomass responses (seed head specific length; mg cm⁻¹) to unshaded controls. We specifically hypothesized that (i) seed head photosynthesis in crested wheatgrass would be consistent with its ability to produce viable seeds, and would have higher A_{sat} but similar Q_{req} as typically occurs in species from high light environments (Hamerlynck and Knapp, 1994), (ii) that shading seed heads would reduce post-anthesis seed head biomass in all three species, as observed in other perennial grasses (Warringa and Marinissen, 1997; Warringa et al., 1998), and (iii) defoliation would reduce reproductive ecophysiological performance and allocation, consistent with previous studies (Anderson and Frank, 2003; Hamerlynck et al., 2016).

2. Materials and methods

2.1. Site description and planting history

Ecophysiological and reproductive productivity measurements for this study were made from May 15 to July 15, 2018 on the USDA Agricultural Research Center Northern Great Basin Experimental Range (NGBER; 119°43'W, 43°29'N), located approximately 70 km west of Burns, OR. The site is situated at 1402 m ASL, with a mean annual

temperature of 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, with approximately 71% of this distributed evenly across the cool season period (November–May), with occasional snowfall over the coldest months.

The plants sampled were selected from adult individuals established in a 14 × 145 m (2030 m²) plot used for previous plant demography studies from 2006 to 2011. Soils at the site are classified as a Vil-Decant-Ratto complex, consisting of well-drained loamy to sandy-loam Aridic Durixerols with an indurated illuvial silica duripan (B_{qm}) beginning at 30–50 cm (Lentz and Simonson, 1986). Seed sources were: *Elymus elymoides* var. Toe Jam Creek from North Basin Seed, Yakima, WA (Lot# NBS-CF5-TJC-1) and Granite Seed, Lehi, UT (Lot# SIHY-39289), *Psuedoroegnaria spicata* var. Anatone from L & H Seeds, Connell, WA (Lot# LHS1D3-445-1) and Granite Seed, Lehi, UT (Lot# AGSP-42452) and *Agropyron cristatum* var. Hycrest (Granite Seed, Lehi, UT AGDE Lot# 33426) and Hycrest II (Bruce Seed Farm, Townsend, MT, Lot# 1480-BBHY9A). Prior to planting, the plot was tilled and all grass and shrub matter removed. The soil was then raked and compacted upon planting, which always occurred the last week of October. All weeds and volunteer remnant grasses and annuals were removed from within the plot and the 10 cm area surrounding planted grasses for three years following seedling emergence and establishment. The area has been kept free of herbivory by rodents and livestock by a perimeter fence since its establishment.

2.2. Field experiments and measurements

2.2.1. Soil moisture monitoring and clipping

In the spring of 2016, 120 individuals (40 plants per each species) ranging in age from 7 to 11 years were selected for study. In October 2017, the site was trenched to install 5TM probes (Decagon Devices, Pullman, WA) to measure concurrent rooting zone volumetric soil moisture (θ) and soil temperature (T_{soil}) at 10 cm depth under 60 plants, with the probes inserted into the wall of a slit-trench into rooting zone of individual plants. At this time, we removed the aboveground biomass to crown height of 60 plants (20 plants per species). Starting April 28, 2018, θ was measured every 4 h, with these observations used to determine daily average θ over the course of gas exchange and reproductive biomass measurements. To ensure some degree of reproductive effort, all plants were watered with 2 L on June 7, 2018 (DOY 158; Fig. 1) 12 days after a large rainfall event increased θ to its seasonal maximum (DOY 146; Fig. 1). Another rainfall occurred 2 days after watering, which was bracketed by our gas exchange sampling and raised θ just prior our seed-head shading experiments (DOY 160; Fig. 1).

2.2.2. Photosynthetic light saturation measurements

Light saturation response curves were generated on pre-anthesis seed heads on 5 clipped and 5 unclipped plants of each species (3 spp x 2 clip × 5 reps = 30 plants total) using a LiCOR 6800 portable photosynthesis unit with an attached red/blue LED light source. We measured light responses on two dates: 6/4/2018 prior to rainfall, and 6/11/2018, two days after supplementing rainfall with 2.0 L for all plants (DOY 155 and 162). The light source red/blue ratio was set to 9:1, with an initial irradiance of 1500 μmol m⁻² s⁻¹ photosynthetic photon flux density (PPFD), which was then reduced incrementally to 1200, 900, 600, 300, 150, 50, and 0 μmol m⁻² s⁻¹ PPFD. Prior to enclosure, seed head width was measured for gas exchange area correction after ensuring the enclosed portion of the seed head was oriented perpendicularly for 3 cm across center of the cuvette. *Agropyron cristatum* seed head areas were estimated by calculating the rectangular projected area (A = 3 cm x seed head width), while *E. elymoides* and *P. spicata* seed head areas were estimated as one half of the area of an open cylinder, using the measured width as the cylinder diameter (A = (π*width)/2) x 3 cm). Tissue temperature was measured with fine-wire thermocouple

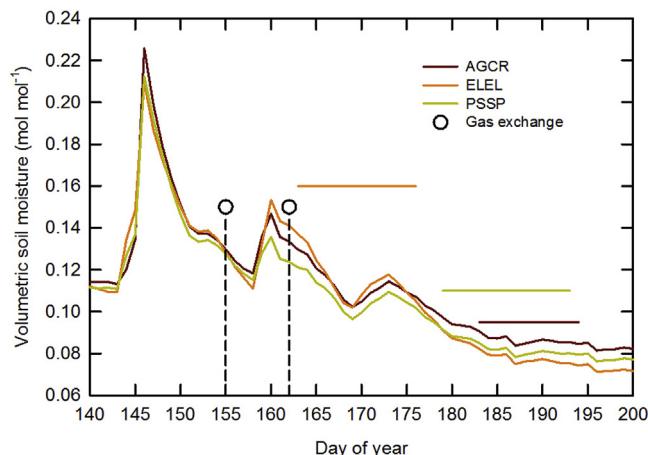


Fig. 1. Rooting-zone volumetric soil moisture of *A. cristatum* (AGCR; crested wheatgrass), *E. elymoides* (EEL; squirreltail wild rye) and *P. spicata* (PSSP; bluebunch wheatgrass) over the gas exchange sampling (circles) and seed head shading experiments; corresponding horizontal colored lines delineate periods over which the respective species seed heads were shaded. Each line is the mean of twenty independent samples, pooled across clipped and unclipped plants.

pressed to underside of the seed head, with the temperature of an attached Peltier cooling block set to 25 °C, and chamber relative humidity set to 25% by automatically passing a portion of the airstream through a column of Stuttgart-masse saturated with de-ionized water. These operating parameters resulted in leaf-to-atmosphere vapor pressure deficits of 1.0–2.8 kPa, depending on prevailing ambient outside air temperature and light conditions. Reference cell CO₂ concentration was set to 400 PPM, with sample cell concentrations slightly lower or higher depending on photosynthetic or respiratory activity. Chamber fan speeds and total airflow rates through the system were adjusted automatically to maintain a cuvette to exterior pressure difference of 0.1 kPa to counteract any leak effects. Enclosed samples were allowed to equilibrate for a minimum of 1 min at each light level, with photosynthetic gas exchange data recorded after signal stability criteria of reference and sample chamber [H₂O] and [CO₂] were met (i.e. < 1% change in signal min⁻¹, with standard deviations less than 1.0). Sampling times at each light intensity ranged from ca. 90 sec to as long as 3 min, depending on the physiological status of the seed head. For low light intensities resulting in small reference-sample concentration differences, reference and sample IRGAs were matched to common conditions prior to recording gas exchange data, following automated matching protocols developed by the instrument manufacturer. We paired immediately adjacent clipped and unclipped control plants of each species and randomly sampled species through the day to reduce diurnal effects on physiological activity across species and clipping treatments. Light saturation curves were analyzed with non-linear regression in Excel (2013) using the SOLVER.xlam add-on, using the model of Ye (2007) to estimate light-saturated net photosynthetic assimilation (A_{sat}) and dark respiration (R_{dark}) rates, photosynthetic light compensation point (I_{comp}) and quantum requirement (Q_{req} ; photons CO₂⁻¹) (Lobo et al., 2013; <https://link.springer.com/article/10.1007/2Fs11099-013-0045-y>).

2.2.3. Seed head shading experiment

Seed heads from plants with more than two flowering culms were randomly selected to be shaded with reflective mylar sheaths ("shaded") or left uncovered as a control ("unshaded"). The mylar sheath reduced full incident PPFD from 2000 μmol m⁻² s⁻¹ to 10–12 μmol m⁻² s⁻¹ when placed over the external quantum sensor of the LiCOR 6800 (Hamerlynck unpublished data). We attempted to have five shaded and unshaded flowering culms per plant; this was not always possible, especially in clipped plants, which in some cases

produced no flowering culms. Seed heads were enclosed at the end of anthesis, and kept in place for a minimum of 10 days (with this period tracked for individual plants). Overall, shading treatments lasted 10–14 days; *E. elymoides* finished anthesis first, and was shaded June 11–25 (DOY 163–176), followed by *P. spicata* (June 28 – July 11; DOY 179–183), and *A. cristatum* (July 2–13; DOY 183–194). After shading, shaded and unshaded seed heads were clipped at the base, measured for length, dried at 40 °C for two days prior to weighing to the nearest 0.0001 g. Seed head specific length (mg cm⁻¹) was determined for each seed head, and averaged for all seed heads per individual plant for statistical analysis.

2.3. Statistical analyses

We used a split-plot repeated-measures ANOVA (RM-ANOVA, general linear models, Statistix v. 8.0, Analytical Software, Tallahassee, FL) to determine the effects of species and clipping treatments on A_{sat} , R_{dark} , I_{comp} and Q_{req} . The whole-plot effects were species, clipping treatment, and the species-by-clipping treatment interaction, using the species-by-clipping treatment-by-replicate plant interaction as the whole-plot error term. Sub-plot, within treatment effects were sampling date (6/04 and 6/11), and all two-way and three-way interactions, using the species-by-clipping treatment-by-date-by-replicate interaction as the sub-plot error term.

Repeated-measures ANOVA was also used to test for differences in θ and seed head specific length. For analysis of θ , we pooled daily average θ over two time periods, first over the entirety of the *E. elymoides* shading period (DOY 162–176), the second over the overlapping time period for *P. spicata* and *A. cristatum* shading periods (DOY 183–192). Species was the whole-plot effect, using the species by replicate interaction effect as the whole-plot error term. The two time periods and the species-by-time period interaction were the sub-plot within-species effects, using the species-by-period-by-replicate interaction as the subplot error term. All θ data were arcsine transformed to meet ANOVA data distribution assumptions (Zar, 1974). To test for effects shading had on seed head specific length, individual split-plot RM-ANOVA were made for each species. Clipping treatment was the whole-plot, between treatment term, using the clipping treatment-by-replicate interaction as the whole-plot error term. Sub-plot, within clipping treatment effects were shading treatment, and the clipping-by-shading treatment interaction, using the clipping-by-shading-by-replicate interaction as the sub-plot error term. To provide a general context for the individual RM-ANOVA results, we ran an additional one-way ANOVA to test for species differences in seed head specific length pooled across clipping and within-plant shading treatments. In all ANOVA analyses, post-hoc means tests of effects sharing the same error term were made using α -adjusted LSD, with an associated p-value of 0.05 considered significant.

3. Results

3.1. Photosynthetic light saturation responses

The three species differed in A_{sat} , R_{dark} , I_{comp} and Q_{req} , with no differences between clipped and unclipped plants, and no interaction effects (Table 1). The two gas exchange rate-based parameters showed significant differences between the two sample dates, with no interaction effects (Table 1). Gas exchange rates were ca. 1.5–2.0 fold higher in *A. cristatum* seed heads ($7.93 \pm 0.628 \mu\text{mol m}^{-2} \text{s}^{-1}$ for A_{sat} and $8.88 \pm 0.534 \mu\text{mol m}^{-2} \text{s}^{-1}$ for R_{dark} , respectively) compared to those in native grass seed heads. Native grass seed heads attained similar A_{sat} pooled across the two sampling dates ($1.98 \pm 0.315 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $1.95 \pm 0.286 \mu\text{mol m}^{-2} \text{s}^{-1}$ for *E. elymoides* and *P. spicata*, respectively) (Fig. 2a), while R_{dark} in *E. elymoides* ($2.39 \pm 0.239 \mu\text{mol m}^{-2} \text{s}^{-1}$) was significantly lower than in *P. spicata* seed heads ($4.15 \pm 0.453 \mu\text{mol m}^{-2} \text{s}^{-1}$; Fig. 2b). The marked declines in A_{sat} and R_{dark} in native grass seed heads were sufficient to result in the

Table 1

Repeated-measures analysis of variance (RM-ANOVA) F-test results comparing species, clipping and sampling date effects on photosynthetic light saturation curve derived parameters of light-saturated net photosynthetic assimilation (A_{sat} ; $\mu\text{mol m}^{-2} \text{s}^{-1}$), dark respiration (R_{dark} ; $\mu\text{mol m}^{-2} \text{s}^{-1}$), photosynthetic light compensation point (I_{comp} ; $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density) and quantum requirement (Q_{req} ; photons CO_2^{-1} fixed). Italic effects and results are within-treatment repeated measures effects; bold F-test results with * = significant at $p \leq 0.05$ and ** significant at $p \leq 0.01$, degrees freedom for each F-test presented parenthetically after effect.

Effect _{dP}	A_{sat}	R_{dark}	I_{comp}	Q_{req}
Species _(2,24)	74.19**	44.97**	3.47*	12.66**
Clipping _(1,24)	2.52 ^{ns}	0.01 ^{ns}	0.24 ^{ns}	1.99 ^{ns}
Spp x Clip _(2,24)	0.84 ^{ns}	0.01 ^{ns}	0.99 ^{ns}	0.33 ^{ns}
Date _(1,24)	4.83*	10.89**	1.62^{ns}	3.33^{ns}
Date x Spp _(2,24)	0.90 ^{ns}	0.01 ^{ns}	0.32 ^{ns}	0.14 ^{ns}
Date x Clip _(1,24)	0.20 ^{ns}	3.06 ^{ns}	0.11 ^{ns}	0.23 ^{ns}
Date x Spp x Clip _(2,24)	0.11 ^{ns}	0.98 ^{ns}	0.46 ^{ns}	0.05 ^{ns}

significant declines from 6/04 to 6/11 pooled across all three species (Table 1) despite more modest declines in *A. cristatum* seed head A_{sat} and R_{dark} (Fig. 2a and b).

Photosynthetic light compensation points (I_{comp}) were high and similar in *A. cristatum* ($228.6 \pm 14.67 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF) and *P. spicata* seed heads ($231.2 \text{ mmol m}^{-2} \text{s}^{-1} \pm 22.91 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF), with *E. elymoides* seed heads having significantly lower compensation points than these ($165.2 \pm 10.60 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF; Fig. 3a). The quantum requirement for CO_2 fixation (Q_{req}) differed between all three species, with *E. elymoides* seed heads having the highest Q_{req} ($41.4 \pm 4.52 \text{ photons CO}_2^{-1}$), followed by *P. spicata* ($28.5 \pm 3.27 \text{ photons CO}_2^{-1}$), with *A. cristatum* seed heads having the lowest Q_{req} ($15.4 \pm 1.15 \text{ photons CO}_2^{-1}$; Fig. 3b).

3.2. Soil moisture and seed head shading

Volumetric soil moisture (θ) did not differ under the three species pooled across the two shading periods (RM ANOVA $F_{2,56} = 1.57$; $p = 0.217$), but did between the two shading periods ($F_{1,56} = 91.97$; $p \leq 0.001$), with a species-by-period interaction ($F_{2,56} = 4.59$; $p = 0.014$). Post-hoc means testing showed θ was higher over the *E. elymoides* shading period ($0.113 \pm 0.0007 \text{ mol mol}^{-1}$) than over the combined *P. spicata/A. cristatum* shading period ($0.083 \pm 0.0006 \text{ mol mol}^{-1}$). The species-by-period interaction resulted from changes in species rankings between the two periods. Over the *E. elymoides* shading period, θ under *A. cristatum* and *E. elymoides* ($0.115 \pm 0.0016 \text{ mol mol}^{-1}$ and $0.116 \pm 0.0014 \text{ mol mol}^{-1}$, respectively) were higher than under *P. spicata* ($0.108 \pm 0.0014 \text{ mol mol}^{-1}$). Over the common *P. spicata/A. cristatum* shading period, θ under *A.*

cristatum ($0.088 \pm 0.0011 \text{ mol mol}^{-1}$) and *P. spicata* ($0.081 \pm 0.0010 \text{ mol mol}^{-1}$) were higher than under *E. elymoides* ($0.077 \pm 0.0012 \text{ mol mol}^{-1}$; Fig. 1).

Seed heads of the three species differed significantly in specific length (One-way ANOVA $F_{2,57} = 361.71$; $p \leq 0.001$), with post-hoc LSD showing *A. cristatum* seed head specific lengths ($28.4 \pm 0.66 \text{ mg cm}^{-1}$) were significantly higher than in *E. elymoides* ($11.3 \pm 0.48 \text{ mg cm}^{-1}$), which in turn were significantly higher than those in *P. spicata* ($5.5 \pm 0.19 \text{ mg cm}^{-1}$). On an individual species basis, clipping and shading did not result in significant differences in seed head specific length for *A. cristatum* and *P. spicata*, but did in *E. elymoides*, with no significant clipping-by-shading interaction in any species (Table 2). Unclipped *E. elymoides* had seed heads with higher specific lengths ($12.7 \pm 0.70 \text{ S.E. mg cm}^{-1}$) than those from clipped plants ($9.8 \pm 0.55 \text{ mg cm}^{-1}$), and unshaded seed heads had higher specific lengths ($12.2 \pm 0.72 \text{ mg cm}^{-1}$) than in shaded seed heads ($10.4 \pm 0.60 \text{ mg cm}^{-1}$). Larger increases between clipped and unclipped control plants in unshaded *E. elymoides* seed heads compared to more modest levels in shaded seed heads likely gave rise to the significant differences between both clipping and shading treatments (Fig. 4). There was a similar trend in *A. cristatum*, but the smaller differences between shaded and unshaded seed heads in unclipped control plants resulted in no significant differences between clipping and shading treatments. *Psuedoroegneria spicata* seed heads effectively had identical specific lengths, regardless of clipping or shading treatment (Fig. 4). Additionally, we compared the number of non-flowering plants across clipped and unclipped plants. *Agropyron cristatum* had one non-flowering plant in each clipping treatment, while *E. elymoides* had three non-flowering clipped plants and one non-flowering control, and *P. spicata* had ten non-flowering clipped plants and none in the twenty controls, resulting in a significant difference in the proportion of non-flowering plants between clipped and unclipped treatments ($\chi^2 = 4.57$; $p = 0.029$, 1df).

4. Discussion

As hypothesized, *Agropyron cristatum* seed heads did have higher light-saturated photosynthetic rates compared to those of the native species, consistent with the ability of this exotic grass to consistently produce viable seed crops. Unexpectedly, the exotic grass also had seed heads with significantly lower Q_{req} , indicating *A. cristatum* seed heads, in addition to having higher photosynthetic capacity, are also more photosynthetically efficient. *Agropyron cristatum*'s higher A_{sat} and lower Q_{req} come at the cost of higher R_{dark} and a high photosynthetic light compensation point (Fig. 3a and b). These distinct photosynthetic characteristics likely have a structural basis, as indicated by the greater specific lengths than in native grass seed heads (Fig. 4). Higher specific length suggests *A. cristatum* has a greater amount of photosynthetically

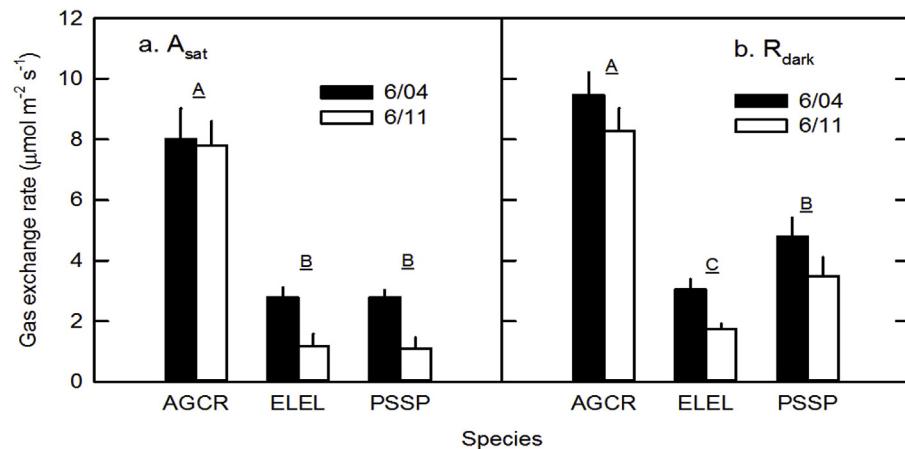


Fig. 2. Light-saturation curve derived estimates of (a) light-saturated net photosynthetic assimilation (A_{sat}) and (b) dark respiration (R_{dark}) rates in seed heads of *A. cristatum* (AGCR; crested wheatgrass), *E. elymoides* (EEL; squirreltail wild rye) and *P. spicata* (PSSP; bluebunch wheatgrass) pooled across clipping treatments over the two sampling dates prior to seed head enclosure. Each bar is the mean of five measurements, error bars are \pm one S.E. of the mean; letters indicate significant differences between species pooled across both sampling dates (LSD from RM-ANOVA; Table 1).

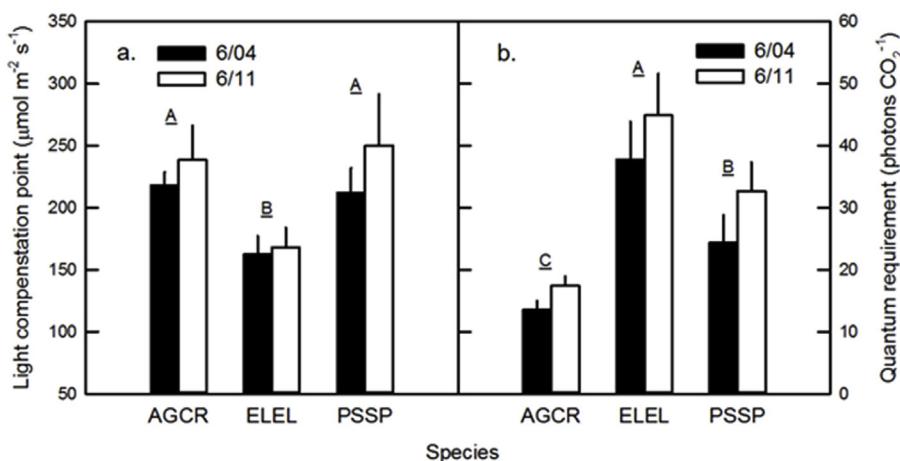


Fig. 3. Light-saturation curve derived estimates of (a) photosynthetic light compensation point (I_{comp}) and (b) quantum requirement of photosynthesis (Q_{req}) in seed heads of *A. cristatum* (AGCR; crested wheatgrass), *E. elymoides* (EEL; squirreltail wild rye) and *P. spicata* (PSSP; bluebunch wheatgrass) pooled across clipping treatments over the two sampling dates prior to seed head enclosure. Each bar is the mean of five measurements, error bars are \pm one S.E. of the mean; letters indicate significant differences between species pooled across both sampling dates (LSD from RM-ANOVA; Table 1).

Table 2

Individual bunchgrass species repeated-measures analysis of variance (RM-ANOVA) F-tests for effects of clipping (clipped and unclipped controls) and seed head shading (shaded and unshaded controls) on seed head specific length ($mg\ m^{-1}$) of crested wheatgrass (*A. cristatum*), squirreltail wild rye (*E. elymoides*) and bluebunch wheatgrass (*P. spicata*). Bold F-test results are significant at $p < 0.05$, degrees freedom (d.f.) for each effect presented parenthetically after each F-test; d.f. vary between species due to differing numbers of non-flowering plants.

Effect	<i>A. cristatum</i>	<i>E. elymoides</i>	<i>P. spicata</i>
Clipping	0.28 _(1,29)	6.90_(1,20)	0.02 _(1,23)
Shading	2.76 _(1,43)	4.80_(1,48)	1.20 _(1,33)
Clip x Shade	0.17 _(1,43)	0.79 _(1,48)	0.02 _(1,33)

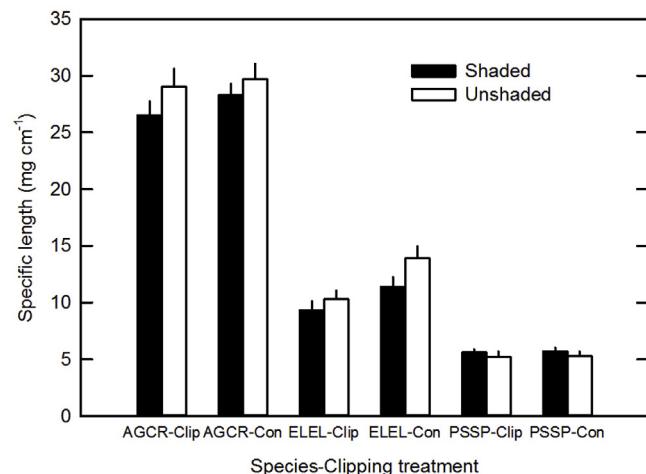


Fig. 4. Specific length ($mg\ cm^{-1}$) of shaded and unshaded seed heads of *A. cristatum* (AGCR; crested wheatgrass), *E. elymoides* (EEL; squirreltail wild rye) and *P. spicata* (PSSP; bluebunch wheatgrass) plants subjected to clipping (-Clip) and unclipped control (-Con) treatments. Each bar is the mean of 10–20 observations (numbers varied with number of non-flowering plants), with bars indicating \pm one S.E. of the mean.

active tissue per seed head, much as leaves with higher specific leaf areas typically have greater photosynthetic capacity and higher dark respiration rates (Hamerlynck and Knapp, 1994; Hamerlynck, 2001; Gonzalez-Paleo and Ravetta, 2018). Producing a denser mass of photosynthetically active inflorescence likely incurs considerable production and maintenance respiration costs in these structures (Amthor, 1984; Thornley, 2011).

Plants in high light environments typically have high

photosynthetic capacity and low light-use efficiency, due to a greater capacity to engage photo-protective mechanisms under excessive light (Demmig-Adams and Adams, 1992; Osmond, 1994; Osmond and Grace, 1995; Pearcy, 1998; Lichtenthaler and Burkart, 1999; Barker et al., 2002; Hamerlynck et al., 2002). Such plants also typically have similar Q_{req} in sun and shade-adapted leaves (Hamerlynck and Knapp, 1994; Hamerlynck, 2001). Sagebrush steppe, like other aridland systems, is not light-limited (Smith et al., 1997). Thus, it is unlikely lower Q_{req} in *A. cristatum* reflects greater seed head shade tolerance compared to those of native grasses (Pearcy, 1998). Intermittent light, however, is a feature common to all ecosystems, and is associated with change in cloud cover before and after rainfall (Burgess, 2009). Possibly, lower Q_{req} facilitates seed head carbon uptake by *A. cristatum* as clouds pass and soil moisture becomes available after rain. To fully assess this, photosynthetic induction kinetics would need to be made to determine how rapidly maximum A_{sat} is regained following varying shading intervals, and how long saturating light exposures need to be to offset carbon losses incurred when light levels are below the photosynthetic compensation points (Roden and Pearcy, 1993; Taylor and Long, 2017), which we should note were high in *A. cristatum* (Fig. 3a).

While there were distinct differences in the photosynthetic light responses between species, clipping did not alter seed head photosynthetic light saturation response parameters (Table 1), contrary to our expectations. Thus, while defoliation can reduce overall reproductive biomass in sagebrush steppe bunchgrasses (Anderson and Frank, 2003; Hamerlynck et al., 2016a), this is not brought about by affecting basic photosynthetic functioning in their reproductive structures. In addition, only one species, *E. elymoides*, showed the hypothesized declines in seed head specific length with shading and clipping (Table 2; Fig. 4). *Elymus elymoides* had low A_{sat} and R_{dark} (Fig. 2a) and low I_{comp} and high Q_{req} compared to levels in *A. cristatum* and *P. spicata* (Fig. 3b). This suggests *E. elymoides* has high light requirements for seed head carbon fixation and may rely more on structures within the inflorescence itself to provide carbon for seed filling. In addition, *E. elymoides* initiated and completed anthesis sooner than *P. spicata* and *A. cristatum* (Fig. 1). *Elymus elymoides* establishes rapidly following disturbance (USDA, 2018), and may initiate and complete reproduction sooner than later seral species (Veenendaal et al., 1996; Ellsworth and Kauffman, 2010). Low specific length and R_{dark} are consistent with lower energetic production costs, with the likely benefit of reduced maintenance respiratory costs in *E. elymoides* compared to the other two grasses (Amthor, 1984; Nagel et al., 2004). Lower energetic costs and rapid early season reproductive development likely allowed *E. elymoides* to use soil moisture when it was most available (Fig. 1). Moreover, the drier soil moisture conditions over the *P. spicata* and *A. cristatum* shading periods (Fig. 1) might have limited the number of seed initiation and filling, and the relatively invariant shaded and unshaded

inflorescence specific lengths in these two species are associated with production of the seed head itself (Fig. 4).

However, our experimental protocol did not remove potential carbon inputs from flag leaves or the culm, both of which can act as reproductive carbon sources in annual cereal grasses (Austin et al., 1982; Warrington and Kreuzer, 1996; Taylor and Long, 2017), but not as much in the one perennial grass studied (Warrington and Kreuzer, 1996; Warrington and Marinissen, 1997; Warrington et al., 1998). It may be that *E. elymoides* is not as efficient as *P. spicata* or *A. cristatum* in carbon transfer from these sources to the seed head, as has been found in wheat varieties that differ in yield (Tambussi et al., 2007). This, along with reduced carbon uptake in the seed head, may have contributed to the stronger declines with shading apparent in *E. elymoides* seed head specific length (Fig. 4). *Agropyron cristatum* and native grasses differ in patterns of aboveground and belowground regrowth in response to defoliation (Hardegree et al., 2016; Denton et al., 2018). It should be noted these grasses also differed in total non-flowering plants in clipped and unclipped treatments. *Agropyron cristatum* and *E. elymoides* had similar numbers of non-flowering plants between treatments, while clipped *P. spicata*, whose seed head specific lengths did not respond to shading or clipping (Fig. 4), had many more plants that did not produce flowering culms, consistent with previous findings (Hamerlynck et al., 2016a). These differences suggest the carbon pool available for translocation and reproductive effort might vary considerably between these grasses, hence the more marked reductions in clipped *E. elymoides* seed head specific lengths.

In summary, the photosynthetic light saturation response characteristics of *A. cristatum* seed heads were consistent with this exotic species ability to readily establish from seed, while our shading experiment indicated carbon sources external to the inflorescence play a role in reproductive effort. Taken in total, these findings strongly suggest these bunchgrasses have distinct suites of traits that shape their reproductive strategies in order to cope with the highly variable climate and disturbance regimes typical of sagebrush steppe ecosystems (Svejcar et al., 2017). Future research using stable isotope or experimental manipulations of vegetative and reproductive structures will need to be made in order to determine the scope of variation in the interactions between vegetative structures and seed head photosynthetic activity in these bunchgrasses (Sanchez-Bragado et al., 2016). Furthermore, carefully assessing sources of variation in reproductive photosynthesis and associated reproductive success in these bunchgrasses will address a basic knowledge gap in the functional ecology of these plants. In the case of native grasses, this information could help selection and development of plant materials with characteristics similar to those natural selection has imparted in the successful exotic. This could enhance the success of post-fire reseeding efforts and the establishment of self-sustaining native grass populations in sagebrush steppe rangeland ecosystems.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jaridenv.2019.104013>.

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