The relationship between seed mass and young-seedling growth and morphology among nine bluebunch wheatgrass populations

Jayanti Ray Mukherjee[1,2], Thomas A. Jones[3], Thomas A. Monaco[3], and Peter B. Adler[2]

[1] Azim Premji University, School of Liberal Studies, Bengaluru, India, [2]formerly Graduate Program, Dept. of Wildland Resources, and Ecology Center, Utah State University, Logan, UT, and [3]USDA-ARS Forage and Range Research Laboratory, Logan, UT.

Abstract:

To better match plant materials to ecological site for purposes of rangeland seedling establishment, we examined the relationship between seed size and growth and morphological traits in young seedlings of bluebunch wheatgrass (BBWG) (*Pseudoroegneria spicata* [Pursh.] Á. Löve), a perennial Triticeae bunchgrass native to the Intermountain West. Traits examined included onset of germination, seedling biomass traits, and seedling surface-area traits. We grew seeds of 9 BBWG populations that varied for seed size and were produced in a common environment under 2 contrasting day/night temperature regimes (20/15 °C; 10/5 °C). Lighter-seeded populations germinated and initiated shoots earlier. Heavier-seeded populations displayed high levels of biomass-related traits, e.g., shoot and root biomass and shoot length, while lighter-seeded populations displayed high levels of surface area-related traits, e.g., specific leaf area and specific root length (SRL). Correlations between seed size and young-seedling traits were mostly similar under the two temperature regimes. However, root length-related traits showed more positive correlations with seed size under the low-temperature regime, which is more similar to actual field-emergence conditions during early spring. P-24, a light-seeded population, originated from the most arid site and exhibited the highest SRL at low temperature, while T-17t, a heavy-seeded population, originated from the most mesic site and exhibited moderate SRL. Three populations used for rangeland revegetation, 'Whitmar,' 'Goldar,' and Anatone Germplasm, all exhibited low seed mass and high SRL. However, only Anatone displayed high root-to-shoot length ratio under both temperature regimes, perhaps explaining its wide and successful use in rangeland seedings.

Key Words: early germination; *Pseudoroegneria spicata*; seed size; specific leaf area, specific root length

Introduction:

Establishment of native perennial Triticeae grasses in rangeland seedings in the Intermountain Region has long lagged that of their Old World relatives, the crested wheatgrasses (*Agropyron* spp.), particularly at sites receiving less than 300 mm of annual precipitation (Asay et al., 2001). One of the most important of these native perennials for restoration purposes is bluebunch wheatgrass (BBWG) (*Pseudoroegneria spicata* [Pursh] Á. Löve) (Jones and Larson, 2005). To improve the success of bluebunch wheatgrass seedings, a better understanding of their seed and seedling functional traits and the potential trade-offs among them may lead to the development of improved plant materials. Such an understanding may also lead to the deployment of specific plant materials to the restoration sites to which they are best adapted (Jones et al., 2010). To be effective, this must be based on a comprehensive understanding of 1) the traits themselves, 2) the expression of the traits among the various plant materials, 3) the edaphic and climatic variables that predominate on specific restoration sites, and 4) the best correspondence between the functional traits of a plant material and the environmental parameters of a site. Seed mass is a trait believed to be one of a suite of traits collectively evolved that has great ecological ramifications across the various life stages of the plant (Moles and Westoby, 2006).

Seed mass is a highly heritable trait (Table 2 in Sadras, 2007) and a major determinant of germination rate, seedling growth, and establishment (Harper et al., 1970; Fenner and Thompson, 2005). Species with larger seeds contain greater reserves and display higher germination percentage, greater emergence from deep seeding, larger initial seedling mass, higher root-extension rates, better seedling establishment, and greater stress tolerance relative to species with smaller seeds (Westoby et al., 1992; Leishman and Westoby, 1994; Kidson and Westoby, 2000; Murray et al., 2004; Baraloto et al., 2005). While much of the high seed-mass advantage occurs between seed production and emergence, additional advantages include greater seedling survival, greater canopy area, and greater adult longevity (Moles and Westoby, 2006). Similar relationships are also known among populations from contrasting environments within species (Kneebone, 1972; Wulff, 1973; Wulff, 1986; Zhang and Muan, 1990; Kitchen and Monsen, 1994; Greipsson and Davy, 1995; Baraloto et al., 2005). For example, across 47 natural populations of BBWG sown at a deep seeding depth (4 cm), seed mass was positively correlated with percentage emergence and seedling shoot mass, though uncorrelated with germination rate, under two temperature regimes (Kitchen and Monsen, 1994).

Small-seededness can also confer traits favorable to seedling growth. An across-continent comparison of species suggests that smaller-seeded species germinate faster (Norden et al., 2009), and their seedlings display greater specific leaf area (SLA) and specific root length (SRL), which are associated with high relative growth rate (RGR) (Wright and Westoby, 1999; Poorter and Garnier, 2007). High RGR allows seedlings grown from smaller seeds to eventually match or surpass the growth of seedlings grown from larger seeds (Thomas, 1966; Westoby et al., 1992; Aparicio et al., 2002). While the negative relationship between seed mass and RGR is well established among species, this relationship is less studied across genotypes within species (Townsend and Wilson, 1981; Meyer and Carlson, 2001; Castro et al., 2008).

Small-seeded species are known to have more stringent temperature requirements for germination than large-seeded species (Easton and Kleindorfer, 2008). Contrasting diurnal temperatures are one of the most critical requirements for germination (Thompson and Grime, 1983; Gutterman, 1993; Liu et al., 2013), and optimal ambient temperature during periods of soil-water availability is an important cue for seed germination (Easton and Kleindorfer, 2008). Temperature may affect initial seedling mass, initial leaf area, and RGR (Townsend and Wilson, 1981), complicating the relationship between seedling morphological traits and their growth rate functions. For example, SLA plays a dominant role in interspecific variability for RGR, though only at higher temperatures (Atkin et al., 2006). However, such information is still lacking for intraspecific comparisons.

Bluebunch wheatgrass is a C_3, perennial Triticeae bunchgrass species that occurs throughout the North American Intermountain West and is commonly included in restoration seed mixes in the region (Monsen et al., 2004). While considerable resources have been devoted to developing more effective plant materials of BBWG (Asay et al., 2003; St. Clair et al., 2013; Gibson and Nelson, 2017), individual plant materials might be better matched to target environments if the ecological implications of seed mass were better understood. In addition, such knowledge would illuminate efforts to intentionally develop BBWG plant materials that are better adapted to specific ecological sites.
Growth of BBWG seedlings at low temperature is important because its germination in the Intermountain West takes place in early spring when temperatures are low but soil-moisture availability is high (Harris and Wilson, 1970). The relatively slow germination of BBWG relative to the prevalent, invasive, exotic winter-annual grasses, and its

inability to preempt abundant early-season soil moisture and nutrient resources from these competitors, has contributed to its decline (Harris, 1967; Young and Allen, 1997).

Our objectives were to assess relationships between seed mass and seedling traits and the response of these relationships to contrasting temperature regimes in order to suggest environmental scenarios for which BBWG genotypes of differing seed mass might be best adapted. To accomplish these objectives, we compared 9 BBWG populations that varied for seed mass for germination traits, seedling growth traits, and seedling morphological traits under 2 day/night temperature regimes (10/5 ^{o}C; 20/15 ^{o}C). To obtain information on BBWG plant materials currently in use, ‘Whitmar,’ ‘Goldar,’ and Anatone Germplasm were included, along with 6 other populations chosen to span a broad range for seed mass.

We tested 3 hypotheses. First, we hypothesized that light-seeded populations would germinate faster than heavy-seeded populations, based on previous findings within species (Maun and Cavers, 1971) and among species (Jurado and Westoby, 1992; Norden et al., 2009) (Hypothesis 1). Next, we hypothesized that heavy-seeded populations would display seedling traits associated with high biomass, e.g., high shoot mass, root mass, and AGR (Hypothesis 2a), while light-seeded populations would display seedling traits associated with high surface area, e.g., high SLA, SRL, and RGR (Hypothesis 2b). Then, we hypothesized that correlations between seed mass and seedling traits would be weaker at low temperature than at high temperature (Hypothesis 3), as cold temperatures delay germination (Gutterman, 1993) and significantly reduce seedling growth (Townsend and Wilson, 1981). Finally, we evaluated seedling growth of three commercially available BBWG plant materials to assess their similarities and differences for seedling traits.

Materials and Methods:

We utilized three released commercial populations (‘Goldar,’ ‘Whitmar,’ and Anatone Germplasm) and six experimental populations (P-3, P-20t, P-22, P-24, P-27t, and T-17t) of BBWG. Of these nine populations, P-20t, P-27t, and T-17t are autotetraploids (2*n* = 28), as indicated by the terminal ‘t’, while the remainder are diploids (2*n* = 14). Both diploid and autotetraploid populations of BBWG occur naturally (Jensen et al., 1995). Of the autotetraploids, T-17t is naturally occurring (Steptoe Butte, Whitman Co., WA), P-27t was developed from an autotetraploid experimentally induced with colchicine from the diploid P-3 (a population developed by intermating several collections in the Grande Ronde Valley in Union Co. and Wallowa Co., OR), and P-20t was derived from a cross between the natural autotetraploid T-17t and an autotetraploid colchicine-induced from P-3. The diploid P-22 population was developed by four cycles of selection on P-1 (origin unknown), and the diploid P-24 population was developed by three cycles of selection on Acc:238 (Lind, WA). All 3 commercial populations trace to collections made from single local sites.

To avoid confounded maternal effects resulting from multiple seed-production environments, seeds of all populations were produced in a common environment at Utah State University’s Evans Farm at Millville, UT. While all seeds were produced in the same field and year, individual seed-production plots were established perpendicular to the prevailing wind and isolated by distance to minimize cross-pollination between plots. Seeds were harvested in 2007, cleaned, and refrigerated until experimental use. Average seed mass was determined by weighing 36 lots of 100 seeds for each population (Fig. 1).

Four seeds were placed in each germination pouch (CYG seed germination pouch, Mega International, St. Paul, MN, USA), which is a folded plastic sheet enclosing a blotter paper. Twenty-five pouches (replicates) of each population were assigned to low and high temperature regimes in separate growth chambers (Percival Scientific, Inc., Perry, IA, USA), in a completely randomized design with subsampling. Thus, a total of 100 seeds of each population were evaluated for each temperature regime. Actual temperatures for low (alternating 12-hour 10 ± 0.33 °C days/ 12-hour 5 ± 0.28 °C nights) and high (20 ± 1.25 °C days/ 15 ± 2.16 °C nights) temperature regimes were recorded in each chamber using a data logger (Watchdog, Spectrum Technologies, Plainfield, IL, USA). The low and high temperature regimes were intended to represent typical early- and late-spring temperatures, respectively, for sagebrush-steppe plant communities of the Intermountain West (West, 1983).

To initiate the study, we sprayed the blotter paper to saturation with distilled water (an equal amount for each germination pouch) on 12 Aug. 2008. Pouches were rewatered as necessary throughout the experiment to maintain saturated conditions. Each seed was monitored daily, and the dates of first initiation of radicle and coleoptile were recorded. Seedlings of 12 replicates were destructively harvested after 8 days for high temperature and 16 days for low temperature (early harvest-date). The low-temperature harvest-date was delayed to allow seedlings to grow large enough to measure morphological traits. For both temperature regimes, a late harvest-date was taken 7 days following the early harvest-date on the remaining 13 replicates to permit calculation of growth rates. On their designated harvest-dates, seedlings were scanned at 300 dots per square inch (46.5 dots per cm^2) using WinRHIZO Pro Version 2005b (Reagent

Instrument Inc., Québec City, Canada). After scanning, shoots and roots were separated, oven-dried at 60 °C for 48 h, and weighed. Scanned images were analyzed for shoot and root length, leaf area, SLA, and SRL. All seedling traits except days to root and shoot initiation were measured at the late harvest-date. Absolute growth rate (AGR) was calculated as $AGR = \mu[(W_2 - W_1)] / (t_2 - t_1)$, and relative growth rate was calculated as $RGR = \mu[(\ln W_2 - \ln W_1)] / (t_2 - t_1)$, where W_1 and W_2 were the total dry masses (shoot + root) from the early and late harvest-dates, respectively, and μ was the mean biomass across replicates for the indicated harvest-date (early or late). As AGR and RGR were calculated from mean values, to determine whether AGR and RGR differed among populations, we performed ANOVA on total biomass at two temperatures and included harvest-date in the model. A significant effect of the harvest-date x population interaction, i.e., population variation for the [(late harvest-date) – (early harvest-date)] difference, for total biomass would indicate that populations were different for AGR. Similarly, a significant effect of the harvest-date x population for ln-transformed total biomass would indicate that populations were different for RGR. Also, a significant interaction for harvest-date x temperature x population for total biomass or ln-transformed biomass would indicate differential variation among populations for either AGR or RGR, respectively, at different temperatures.

Data were analyzed with SAS 9.2 (SAS Institute 2003). Using PROC MIXED, we employed a sequential analysis-of-variance model with ploidy fitted first, followed by seed mass, and then populations. All independent variables were considered fixed, except for replicate, which was considered random. We used data from all 25 replicates to calculate days to germination, days to shoot initiation, AGR, and RGR, while the remaining morphological traits were determined from the 13 replicates measured at the late harvest-date. Data transformation was performed as necessary to meet normality assumptions. Means were back-transformed for presentation.

Using PROC CORR, we calculated unadjusted Pearson’s correlation coefficients between seed mass and 1) days to germination and 2) days to shoot initiation to test Hypothesis 1. Similarly, we calculated correlation coefficients between seed mass and 1) shoot mass, 2) root mass, and 3) AGR to test Hypothesis 2a and between seed mass and 1) SLA, 2) SRL, and 3) RGR to test Hypothesis 2b. Next, we estimated correlation coefficients between seed mass and 11 seedling traits (not including RGR) at high and low temperatures. We then tested Hypothesis 3 by determining if the seed mass x temperature interaction was significant for each of 12 seedling traits.

Results:

We used 9 BBWG populations, spanning a broad range for seed mass, to test our 3 hypotheses involving the effects of seed mass on young-seedling growth. Six of the populations were diploid, but the 3 others were autotetraploid. Seed mass ranged from 6.46 mg seed^{-1} for the autotetraploid P-27t to 3.48 mg seed^{-1} for the diploid Whitmar, a 1.9-fold range. On average, autotetraploid populations had heavier seeds than diploid populations (5.99 vs. 4.33 mg seed^{-1}), though an exception to this general trend was the high seed mass (6.18 mg seed^{-1}) diploid, P-3. These two variables, ploidy and seed mass, displayed a high degree of multicollinearity in our data set, making it impossible to separate their confounded effects. Because we didn't have an adequate experimental design or plant materials to test differences between the 2 ploidies, we used ploidy only as a covariate in the ANOVA to control for any ploidy effects. Subsequent to ploidy, seed mass, population, temperature, and their interactions were fitted in the model. Thus, our hypotheses relate to seed mass, population (residual after seed mass), and the temperature x seed mass interaction effects rather than to ploidy.

For some traits, we found residual variation among populations after ploidy and seed mass variation were accounted for, but not for others (Table 1). Residual variation among populations was found for 5 traits (days to root and shoot initiation, root biomass, root and shoot length), but in each of these cases most variation was accounted for by either ploidy or seed mass. For 5 other traits (shoot biomass, root:shoot length ratio, SRL, SLA, AGR), no residual variation remained after ploidy and seed mass were accounted for. Only for root:shoot biomass ratio was the population effect significant and ploidy and seed mass effects non-significant.

The two growth-rate variables, AGR and RGR, were necessarily calculated by determining the interaction of biomass across the 2 harvest-dates. We found a significant harvest-date x population interaction for total biomass ($P < 0.005$) but none ($P > 0.10$) for ln-transformed total biomass, indicating that populations differed for AGR but not for RGR. Absolute growth rate and RGR were calculated separately for each temperature.

Supporting Hypothesis 1, small-seeded populations germinated faster, as seed mass was positively correlated at high temperature with days to root initiation ($r = 0.72$; $P < 0.05$) (Fig. 2a) and days to shoot initiation ($r = 0.67$; $P < 0.05$) (Fig. 2b). In accordance with Hypothesis 2a, seed mass was positively correlated across populations and temperatures with biomass traits, e.g., shoot mass ($r = 0.83$; $P < 0.01$), root mass ($r = 0.82$; $P < 0.01$), AGR ($r = 0.74$; $P < 0.05$), and shoot length ($r = 0.67$; $P < 0.10$) (Table 2, Fig. 3a). In accordance with Hypothesis 2b, seed mass was negatively correlated across populations and temperatures with surface-area traits, e.g., SLA ($r = -0.73$; $P < 0.05$) (Table 2, Fig. 3b) and SRL ($r = -0.90$; $P < 0.01$) (Table 2, Fig. 3c). Thus, low seed mass was associated with high SLA and high SRL. However, RGR, a trait often associated with high surface area was not correlated with seed mass, as RGR was not found to vary ($P > 0.10$) among populations. Correlations between AGR and individual seedling traits were similar to correlations between seed mass and those traits, likely because of the positive correlation between seed mass and AGR ($r = 0.74$; $P < 0.05$) (Table 2). Like high seed mass, high AGR was associated with low surface-to-mass ratio, as evidenced by negative correlations between AGR and SLA ($r = -0.64$; $P < 0.10$) (Fig. 4a) and SRL ($r = -0.73$; $P < 0.05$) (Fig. 4b). The lack of significance for the harvest-date x temperature x population interaction indicates that populations varied similarly across temperatures for AGR. On average, P-20t (0.299 mg day^{-1}) had highest AGR, while Whitmar was lowest (0.204 mg day^{-1}).

We used the significance of the seed mass x temperature correlation to assess whether the relationship between seed mass and seedling traits varied between temperature regimes (Table 2). We found no evidence of a difference between temperature regimes for seed mass correlations with 8 of the traits, as the temperature x seed mass interaction was not significant ($P > 0.10$). Thus, our data failed to support Hypothesis 3 for these traits. However, temperature did change the relationships of seed mass with three traits related to seedling root length. A temperature x seed mass interaction was found for root length ($P < 0.0001$), R:S length ratio ($P < 0.0001$), and SRL ($P < 0.05$) (Table 1). In each case, the correlation of each trait with seed mass was more positive at low temperature than at high temperature, though the sign of the correlation remained negative for SRL. This negative correlation between seed mass and SRL is explained by the fact that the positive relationship between seed mass and root biomass was stronger than the positive relationship between seed mass and root length. To summarize, our data suggest that high seed mass was more associated with greater root length under the low-temperature than under the high-temperature regime.

Discussion:

The ability to germinate and successfully establish is paramount for ecological fitness. Larger seeds within a grass species often generate seedlings of greater vigor (Rogler, 1954; Kittock and Patterson, 1962; Kneebone, 1972; Smith et al., 2003), and in our study, heavier-seeded populations produced larger seedlings. However, because large seeds tend to produce seedlings with high AGR but low RGR, over time seedlings of small seeds may match or surpass the size of seedlings of large seeds (Thomas, 1966; Aparicio et al., 2002). Furthermore, a trade-off may occur between seed mass, which is primarily under genetic control, and seed number, which is primarily under environmental control (Cloé and Turnbull, 2009). However, these authors showed that this trade-off may not be one-for-one if adult size, resource availability, or competitive ability differ among genotypes. When nutrients were highly limiting, *Arabidopsis thaliana* genotypes with low seed mass were unable to completely compensate by increasing seed number, so large-seeded genotypes possessed greater ecological fitness (overall yield) (Cloé and Turnbull, 2009). On the other hand, when nutrients were plentiful, small-seeded genotypes were more than able to compensate by increasing seed number, giving them greater fitness (Alonso-Blanco et al., 1999, as cited by Cloé and Turnbull, 2009). Thus, the magnitude of the trade-off, and consequently the relative contribution of seed mass vs. seed number to overall ecological fitness, may not be fixed but may vary with environmental conditions and plant traits that impact growth (Cloé and Turnbull, 2009).

Seed mass was long thought of as a trade-off between two opposing evolutionary forces affecting fitness, one force favoring small seeds, due to the favorable effect of increased seed number, and another force favoring large seeds, due to their greater likelihood of growing into established seedlings (Westoby et al., 1992). However, because seed mass may span orders of magnitude across species at a single site, some authors have postulated that a simple trade-off between seed mass and number cannot explain variation for seed mass (Westoby et al., 1992; Moles and Westoby, 2006). Instead, empirical data suggest that seed mass evolves as a component of a suite of life-history traits including length of the juvenile period, juvenile survival rate, mature-plant size, and plant longevity (Westoby et al., 1992; Moles and Westoby, 2006). Categories of growth form (graminoid, forb, shrub, tree, or vine) correlate with seed mass both among extant species and in the fossil record (Moles et al., 2005). Moles et al. (2005, 2007) have suggested that large seed mass is associated with large mature-plant size because the latter requires a longer juvenile period, and longer juvenile periods necessitate high seedling survival, which in turn is favored by larger seed mass.

Within a species, seed mass may be reflective of site adaptation, being associated with specific abiotic and biotic parameters that characterize groups of environmentally similar sites. For example, among 1,600 provenances of 34 Australian perennial species of *Glycine*, seed mass was higher at lower latitudes and in the interior of Australia. Higher seed mass was most closely associated with higher temperature and solar radiation, as opposed to differences in precipitation (Murray et al., 2004). In this (Murray et al., 2004) and another study (Moles and Westoby, 2006), patterns among and within species were generally consistent. Heavier seeds may result from higher photosynthate production due to higher solar radiation, and they may be required in warm climates where metabolic requirements for growth and maintenance are high (Murray et al., 2004).

In fact, migrations away from the equator have been associated with declines in seed mass throughout seed-plant evolution (Moles et al., 2007).

Most intraspecific studies of the relationship between seed mass and germination have examined variation within rather than among populations. Variation for seed mass within populations has been generated by either manually sorting seeds, e.g., Winn (1985), Wulff (1986), Paz and Martínez-Ramos (2003), or by utilizing half-sib open-pollinated lines that vary for seed mass, e.g., Benard and Toft (2007), Castro et al. (2008). Among-population studies have generally used seeds collected from wildland sites, e.g., Counts and Lee (1991), Kitchen and Monsen (1994), Greipsson and Davy (1995), Meyer and Carlson (2001), thereby introducing a confounding maternal effect due to differences in seed-production environment (Roach and Wulff, 1987). For our among-population study, we intentionally used seeds produced in a common environment to avoid this confounding effect.

Supporting Hypothesis 1, we found that lighter-seeded diploid BBWG populations germinated faster ($r = 0.60$; $P < 0.10$) than both heavier-seeded diploid and autotetraploid populations across temperature regimes (Table 2), though the difference was slight. Kitchen and Monsen (1994) also reported faster germination of lighter-seeded BBWG populations, both at 1 °C ($r = 0.37$; $P < 0.05$) and at 15/25 °C ($r = 0.26$; $P < 0.05$). When soil nutrients and minerals are readily available after precipitation events, it is likely that early germination associated with lighter seeds is desirable so that seedlings may quickly establish. While larger seeds may be slower to establish, their resultant seedlings are generally more tolerant of stress and survive at a higher frequency (Moles and Westoby, 2006; Eliášová and Műnzbergová, 2014). On the other hand, lighter seeds can be produced in larger quantities than heavier seeds, which may allow them to persist in the population despite lower stress tolerance and survival frequencies (Jakobsson and Eriksson, 2000; Moles and Westoby, 2006).

In agreement with Hypothesis 2a, across temperature regimes we found that heavier-seeded populations had greater AGR ($r = 0.74$; $P < 0.05$) (Table 2), a variable that reflects biomass production over time irrespective of initial biomass (Hunt 1990). Heavier-seeded populations displayed higher shoot biomass, root biomass, and AGR, likely because of their greater seed reserves (Fenner and Thompson, 2005). In our study, the 85.6 % difference in seed mass between the lightest-seeded population, Whitmar, and the heaviest-seeded population, P-27t, corresponded to a 68.9 % difference in AGR. Similar to our study, Kitchen and Monsen (1994) reported that seeds of heavier-seeded BBWG populations produced greater seedling shoot biomass ($r = 0.63$; $P < 0.01$).

Relative growth rate is often regarded as a more appropriate variable for population comparisons than AGR (Hunt, 1990). Unlike AGR, RGR, the growth rate per unit of initial biomass, did not vary among our populations, though this may be due to larger standard errors for RGR due to its more complex calculation. Consequently, we found no association between seed mass and RGR, similar to previous intraspecific studies in other species (Wulff, 1986; Tamet et al., 1996; Castro et al., 2008), but different from an interspecific trial ($r = -0.31$; $P < 0.05$) (Larson et al., 2016). At the young-seedling stage, seed mass may be the major factor contributing to early growth (Cideciyan and Malloch, 1982), hence we were able to detect significant variation for AGR. However, the effect of seed mass on seedling size typically diminishes over time, and the size discrepancy between seedlings of heavier and lighter seeds declines with age

(Kittock and Patterson, 1962; Zhang and Muan, 1990; Smart and Moser, 1999). This is the time when variability in RGR becomes more important relative to AGR (Westoby et al, 1992). Thus, our inability to detect differences in RGR could also be a consequence of the young-seedling stage at which our measurements were taken.

Seed mass was negatively associated with SLA and SRL, in agreement with Hypothesis 2b. It appears that lighter-seeded populations invest preferentially in surface area, while heavier-seeded populations invest more in biomass per unit of surface area. This pattern is similar to that reported for interspecific comparisons by Lambers et al. (1998) and Reich et al. (1998), though no relationship was reported by Larson et al. (2015). The negative relationship between seed mass and surface area, either SLA or SRL, suggests that light-seeded populations may be better suited for above- and below-ground resource acquisition in nutrient-rich environments (Westoby, 1998; Poorter and Jong, 1999). Alternatively, species or populations that invest more in biomass and display low SLA and SRL have low nutrient-acquisition rates and generally originate in nutrient-poor environments (Reich et al., 2003). It must be emphasized that the above-cited studies refer to distribution of species across environments that vary for nutrient resources, particularly N, and rarely refer to environmental variation for soil-moisture availability (Westoby et al., 1998). Both higher and lower SRL have been associated with drought-stress environments (Nicotra et al., 2002; Poot and Lambers, 2003)).

Shoot and root biomass and shoot and root length of P-3, the heaviest-seeded diploid, were more similar to the other autotetraploids than to the remaining diploids. This suggests that seed mass rather than ploidy per se is the more important determinant of biomass traits. Both seed mass and higher ploidy have been reported to be contributors to seedling biomass in orchardgrass (*Dactylis glomerata* L.) (Bretagnolle et al., 1995).

Contradicting Hypothesis 3, correlations between seed mass and seedling growth and morphological traits were similar under the 2 temperature regimes. However, of greater interest is the disparity between the 2 temperature regimes for the 3 traits related to root length. The more positive correlations of seed mass and root length-related traits under the low-temperature regime are particularly relevant because they reflect the early-spring environment when perennial seeds are germinating. These data may also explain earlier reports that large seed mass may enhance seedling establishment under drought conditions (Murray et al., 2004).

Because of their use in rangeland seedings, we included three commercially available BBWG populations (Goldar, Anatone Germplasm, and Whitmar) in our study. All displayed low seed mass, high SRL (Fig. 2b), and low AGR (Fig. 2c). However, of these three, only Anatone Germplasm exhibited high R:S length ratio at both temperatures (Fig. 4). The ability of Anatone to display a high R:S length ratio across a broad temperature range might explain the wide and successful use of this plant material in rangeland seedings. The other two populations displayed high values at high temperature but low values at low temperature, the latter being more similar to temperatures to which young seedlings are typically exposed within the species' natural distribution (Harris and Wilson, 1970).

In our study, heavier seeds were associated with lower SRL across populations, though seed mass was less closely related to root length (Table 2). Whether low SRL is advantageous or disadvantageous under our semi-arid conditions is unclear (Ryser, 2006). While our study was not designed to answer this question, we nevertheless note that the light-seeded P-24 originated from the most arid (253 mm average annual precipitation [AAP]) and lowest-elevation (480 m) site, while the heavy-seeded T-17t originated from the most mesic (639 mm AAP) and highest-elevation (1100 m) site. This distinction suggests low seed mass is desirable in arid regions, but this may be a consequence of the impracticality of producing heavy seeds in an arid environment. For example, this could result in a significant trade-off with seed number, which might be evolutionarily disadvantageous overall (Aarssen et al., 2006; Chambers and Aarssen, 2009).

The two above-mentioned accessions, P-24 and T-17t, originating in contrasting wildland sites, had similar SRL values at high temperature. However, at low temperature P-24, the accession originating in the more arid site, featured the highest SRL, while T-17t displayed an intermediate value (Fig. 2b). If high SRL at low temperature, as displayed by P-24, is advantageous for more-arid sites, then high-SRL light-seeded populations may be preferred for successful seedling establishment in such drought-prone environments. In support of this premise, Kulpa and Leger (2013) found that bottlebrush squirreltail (*Elymus elymoides* [Raf.] Swezey ssp. *californicus*) seedlings that successfully established tended to produce smaller seeds.

Credible assessments of site adaptation are best achieved through common-garden studies that measure establishment and persistence and are heavily replicated across time and space (Robins et al., 2013). Seed mass is an easily-measured parameter, but it is only one of many functional traits that impact ecological fitness. Studies cited herein document positive relationships between seed mass and temperature and solar radiation

(Murray et al., 2004; Moles and Westoby, 2006) and negative relationships between seed mass and soil-N concentration (Westoby, 1998; Poorter and Jong, 1999; Cloé and Turnbull, 2009). Therefore, a worthwhile future experiment would be to test BBWG accessions of varying seed mass across sites that span a range of ambient temperatures and soil-N concentrations. Such experiments would need to statistically control for other environmental variables, and data would need to be collected throughout the life-history of the stand to determine at which life-stages seed mass is most closely associated with adaptation.

Implications:

Measurement of seed mass may offer a useful way to characterize populations along a continuum for the suite of 8 traits with which it is correlated. We note that seed mass must be measured on seeds produced in a common environment for it to strictly reflect genotype rather than being confounded with seed-production environment. Our data suggest that lighter-seeded populations will display faster germination and higher surface area (indicative of high RGR), while heavier-seed populations will display slower germination and higher biomass (indicative of high AGR). Seed mass seems to be a key trait that is closely related to young-seedling growth, and we presume that this trait has adaptive value, meaning that optimal seed mass varies with the environment in which the seed is growing. Differences among environments may relate to differences for site and site history variables, e.g., weather, soil, vegetation, previous cultivation, grazing history, fire history. Future experiments may indicate how a plant material with a particular seed mass may be matched to the seedbed environment to which it is best adapted.

Acknowledgements:

We thank Ronald J. Ryel (deceased) and Christopher A. Call for their fruitful comments and intriguing discussions. We also thank Dale Nielson, Jenny Christiansen, and Tren Hagman for their technical help throughout the project and Susan Durham for her assistance with statistical analysis.

References:

Aarssen, L.W., Schamp, B.S., Pither, J., 2006. Why are there so many small plants? Implications for species coexistence. J. Ecol. 94, 569-580.

Alonso-Blanco, C., Blankestijn-DeVries, H., Hanhart, C.J., Koornneef, M., 1999. Natural allelic variation at seed size loci in relation to other life history traits of *Arabidopsis thaliana*. Proc. Nat. Acad. Sci. USA 96, 4710-4717.

Aparicio, N., Villegas, D., Araus, J.L., Blanco, R., Royo, C., 2002. Seedling development by biomass as affected by size and morphology in durum wheat. J. Agric. Sci. 139, 143-150.

Asay, K.H., Chatterton, N.J., Jensen, K.B., Jones, T.A., Waldron, B.L., Horton, W.H., 2003. Breeding improved grasses for semiarid rangelands. Arid Land Res. Manage. 17, 469-478.

Asay, K.H., Horton, W.H., Jensen, K.B., Palazzo, A.J., 2001. Merits of native and introduced Triticeae grasses on semiarid rangelands. Can. J. Plant Sci. 81, 45-52.

Atkin, O.K., Loveys, B.R., Atkinson, L.J., Pons, T.L., 2006. Phenotypic plasticity and growth temperature: understanding interspecific variability. J. Exper. Bot. 57, 267-281.

Baraloto, C., Forget, P.M., Goldberg, D.E., 2005. Seed mass, seedling size, and neotropical tree seedling establishment. J. Ecol. 93, 1156-1166.

Benard R.B., Toft, C.A., 2007. Effect of seed size on seedling performance in a long-lived desert perennial shrub (*Ericameria nauseosa*: Asteraceae). Int. J. Plant Sci. 168, 1027-1033.

Bretagnolle, F., Thompson, J.D., Lumaret, R., 1995. The influence of seed size variation on seed germination and seedling vigour in diploid and tetraploid *Dactylis glomerata* L. Ann. Bot. 76, 607-615.

Castro, J., Reich, P.B., Sánchez-Miranda, A., Guerrero, J.D., 2008. Evidence that the negative relationship between seed mass and relative growth rate is not physiological but linked to species identity: a within-family analysis of Scots pine. Tree Physiol. 28, 1077-1082.

Chambers, J., Aarssen, L.W., 2009. Offspring for the next generation: most are produced by small plants within herbaceous populations. Evol. Ecol. 23, 737-751.

Cideciyan, M.A., Malloch, A.J.C., 1982. Effect of seed size on the germination, growth, and competitive ability of *Rumex crispus* and *Rumex obtusifolius*. J. Ecol. 70, 227-232.

Cloé, P.-V., Turnbull, L.A., 2009. The effect of growth conditions on the seed size/number trade-off. PLoS ONE 4, e6917.

Counts, R.L., Lee, P.F., 1991. Germination and early seedling growth in some northern wild rice (*Zizania palustris*) populations differing in seed size. Can. J. Bot. 69, 689-696.

Easton, L.C., Kleindorfer, S., 2008. Interaction effects of seed mass and temperature on germination of Australian species of *Frankenia* (Frankeniaceae). Folia Geobotanica 43, 383-396.

Eliášová, A., Mὒnzbergová, Z., 2014. Higher seed size and germination rate may favour autotetraploids of *Vicia cracca* L. (Fabaceae). Biol. J. Linnean Soc. 113, 57-73.

Fenner, M., Thompson, K., 2005. The ecology of seeds. Cambridge University Press, Cambridge, U.K.

Gibson, A., Nelson, C.R., 2017. Comparing provisional seed transfer zone strategies for a commonly seeded grass, *Pseudoroegneria spicata*. Nat. Areas J. 37, 188-199.

Greipsson, S., Davy, J., 1995. Seed mass and germination behavior in populations of the dune-building grass *Leymus arenarius*. Ann. Bot. 76, 493-501.

Gutterman, Y., 1993. Seed germination in desert plants. Springer-Verlag, Heidelberg, Berlin.

Harper, J.L., Lovell, P.H., Moore, K.G., 1970. The shapes and size of seeds. Ann. Rev. Ecol. System. 1, 327-356.

Harris, G.A., 1967. Some competitive relationships between *Agropyron spicatum* and *Bromus tectorum*. Ecol. Monogr. 37, 89-111.

Harris, G.A., Wilson, A., 1970. Competition for moisture among seedlings of annual and perennial grasses as influenced by root elongation at low temperature. Ecology 51, 530-534.

Hunt, R., 1990. Basic growth analysis: plant growth analysis for beginners. Kluwer Academic Publishers, Unwin Hyman, London, U.K.

Jakobsson, A., Eriksson, O., 2000. A comparative study of seed number, seed size, seedling size and recruitment in grassland plants. Oikos 88, 494-502.

Jensen, K., Curto, M., Asay, K., 1995. Cytogenetics of Eurasian bluebunch wheatgrass and their relationship to North American bluebunch and thickspike wheatgrass. Crop Sci. 35, 1157-1162.

Jones, T.A., Larson, S.R., 2005. Status and use of important native grasses adapted to sagebrush communities. p. 49-55 In: Monsen, S.B., Shaw, N.L., Pellant, M.

(comps.) Sage-grouse habitat restoration symposium; 2001 July 4-7; Boise, ID. Proceedings RMRS-P-38. Ogden, UT: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

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.

Jurado, E., Westoby, M., 1992. Seedling growth in relation to seed size among species of arid Australia. J. Ecol. 80, 407-416.

Kidson, R., Westoby, M., 2000. Seed mass and seedling dimensions in relation to seedling establishment. Oecologia 125, 11-17.

Kitchen, S.G., Monsen, S.B., 1994. Germination rate and emergence success in bluebunch wheatgrass. J. Range Manage. 47, 145-150.

Kittock, D.L., Patterson, J.K., 1962. Seed size effects on performance of dryland grasses. Agron. J. 54, 277-278.

Kneebone, W.R., 1972. Breeding for seedling vigor, in: Younger, V.B., McKell, C.M. (Eds.), The biology and utilization of grasses. Academic Press, New York, pp. 90-100.

Kulpa, S.M., Leger, E.A., 2013. Strong natural selection during plant restoration favors an unexpected suite of plant traits. Evol. App. 6, 510-523.

Lambers, H., Chapin, S.F., Pons, T.L., 1998. Plant physiological ecology. Springer, New York.

Larson, J.E., Sheley, R.L., Hardegree, S.P., Doescher, P.S., James, J.J., 2015. Seed and seedling traits affecting critical life stage transitions and recruitment outcomes in dryland grasses. J. Appl. Ecol. 52, 199-209.

Larson, J.E., Sheley, R.L., Hardegree, S.P., Doescher, P.S., James, J.J., 2016. Do key dimensions of seed and seedling functional trait variation capture variation in recruitment probability? Oecologia 181, 39-53.

Leishman, M.R., Westoby, M., 1994. The role of seed size in seedling establishment in dry soil conditions – experimental evidence from semi-arid species. J. Ecol. 82, 249-258.

Liu, K., Baskin, J.M., Baskin, C.C., Bu, H.Y., Du, G.Z., Ma, M.J., 2013. Effect of diurnal fluctuating versus constant temperatures on germination of 445 species from the eastern Tibet Plateau. PLoS ONE 8, e69364.

Maun, M.A., Cavers, P.B., 1971. Seed production and dormancy in *Rumex crispus.* I. The effects of removal of cauline leaves at anthesis. Can. J. Bot. 49, 1123-1130.

Meyer, S.E., Carlson, S.L., 2001. Achene mass variation in *Ericameria nauseosus* (Asteraceae) in relation to dispersal ability and seedling fitness. Funct. Ecol. 15, 274-281.

Moles, A.T., Westoby, M., 2006. Seed size and plant strategy across the whole life cycle. Oikos 113, 91-105.

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. 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, 109-226.

Monsen, S.B., Stevens, R., Shaw, N.L., 2004. Restoring western ranges and wildlands. U.S. Forest Service Gen. Tech. Rep. RMRS-GTR 136 (Vol. 2), Fort Collins, CO.

Murray, B.R., Brown, A.H.D., Dickman, C.R., Crowther, M.S., 2004. Geographical gradients in seed mass in relation to climate. J. Biogeogr. 31, 379-388.

Nicotra, A.B., Babicka, N., Westoby, M., 2002. Seedling root anatomy and morphology: an examination of ecological differentiation with rainfall using phylogenetically independent contrasts. Oecologia 130, 136-145.

Norden, N., Davis, M.L., Antoine, C., Gonzalez, M.A., Garwood, N.C., Chave, J., 2009. The relationship between seed mass and mean time to germination for 1037 tree species across five tropical forests. Funct. Ecol. 23, 203-210.

Paz, H., Martínez-Ramos, M., 2003. Seed mass and seedling performance between eight species of *Psychotria* (Rubiaceae). Ecology 84, 439-450.

Poorter, H., Garnier, E., 2007. Ecological significance of inherent variation in relative growth rate and its components, in: Pugnaire, F.I., Valladares, F. (Eds.), Functional plant ecology. CRC Press, Boca Raton, FL, pp. 67-100.

Poorter, H., Jong, R.D., 1999. A comparison of specific leaf area, chemical composition and leaf construction costs of field plants from 15 habitats differing in productivity. New Phytol. 143, 163-176.

Poot, P., Lambers, H., 2003. Are trade-offs in allocation pattern and root morphology related to species abundance? A congeneric comparison between rare and common species in the south-western Australian flora. J. Ecol. 91, 58-67.

Reich, P.B., Tjoelker, M.G., Walters, M.B., Vanderklein, D.W., Buschena, C., 1998. Close association of RGR, leaf and root morphology, seed mass and shade tolerance in seedlings of nine boreal tree species grown in high and low light. Function. Ecol. 12, 327-338.

Reich, P.B., Wright, I.J., Cavender-Bares, J., Craine, J.M., Oleksyn, J., Westoby, M., Walters, M.B., 2003. The evolution of plant functional variation: traits, spectra, and strategy. Intern. J. Plant Sci. 164, S143-S164.

Roach, D.A., Wulff, R.D., 1987. Maternal effects in plants. Ann. Rev. Ecol. System. 18, 209-235.

Robins, J.G., Jensen, K.B., Jones, T.A., Waldron, B.L., Peel, M.D., Rigby, C.W., Vogel, K.P., Mitchell, R.E., Palazzo, A.J., Cary, T.J., 2013. Stand establishment and persistence of perennial cool-season grasses in the Intermountain West and the Central and Northern Great Plains. Rangel. Ecol. Manage. 66, 181-190.

Rogler, G.A., 1954. Seed size and seedling vigor in crested wheatgrass. Agron. J. 46, 216-220.

Ryser, P., 2006. The mysterious root length. Plant Soil 286, 1-6.

Sadras, B., 2007. Evolutionary aspects of the trade-off between seed size and number in crops. Field Crops Res. 100, 125-138.

SAS Institute, 2003. SAS/STAT user’s guide, version 9.1. SAS Institute, Cary, N.C.

Smart, A.J., Moser, L.E., 1999. Switchgrass seedling development as affected by seed size. Agron. J. 91, 335-338.

Smith, K.F., McFarlane, N.M., Croft, V.M., Trigg, P.J., Kearney, G.A., 2003. The effects of ploidy and seed mass on the emergence and early vigour of perennial ryegrass (*Lolium perenne* L.) cultivars. Austr. J. Exp. Agric. 43, 481-486.

St. Clair, J.B., Kilkenny, F.F., Johnson, R.C., Shaw, N.L., Weaver, B., 2013. Genetic variation in adaptive traits and seed transfer zones for *Pseudoroegneria spicata* (bluebunch wheatgrass) in the northwestern United States. Evol. App. 6, 933-948.

Tamet, V., Boiffin, J., Dürr, C., Souty, N., 1996. Emergence and early growth of an epigeal seedling (*Daucus carota* L.): Influence of soil temperature, sowing depth, soil crusting and seed mass. Soil Till. Res. 40, 25-38.

Thomas, R.L., 1966. The influence of seed weight on seedling vigor in *Lolium perenne*. Ann. Bot. 30, 111-121.

Thompson, K., Grime, J.P., 1983. A comparative study on germination responses of diurnally-fluctuating temperatures. J. Appl. Ecol. 20, 141-156.

Townsend, C.E., Wilson, A.M., 1981. Seedling growth of cicer milkvetch as affected by seed weight and temperature regime. Crop Sci. 21, 405-409.

West, N.E., 1983. Western Intermountain sagebrush steppe, in: West, N.E. (Ed.), Temperate deserts and semi-deserts. Elsevier, Amsterdam, The Netherlands, pp. 351-374.

Westoby, M., 1998. A leaf-height-seed (LHS) plant ecology strategy scheme. Plant Soil 199, 213-227.

Westoby, M., Jurado, E., Leishman, M., 1992. Comparative evolutionary ecology of seed size. Trends Ecol. Environ. 7, 368-372.

Winn, A.A., 1985. The effects of seed size and microsite on seedling emergence in four populations of *Prunella vulgaris*. J. Ecol. 73, 831-840.

Wright, I., Westoby, M., 1999. Differences in seedling growth behavior among species: Trait correlation across species, and trait shifts along nutrient compared to rainfall gradients. J. Ecol. 87, 85-97.

Wulff, R.D., 1973. Intrapopulational variation in the germination of seeds in *Hyptis suaveolens*. Ecol. 54, 646-649.

Wulff, R.D., 1986. Seed size variation in *Desmodium paniculatum*: II. Effects on seedling growth and physiological performance. J. Ecol. 74, 99-114.

Young, J.A., Allen, F.L., 1997. Cheatgrass and range science: 1930–1950. J. Range Manage. 50, 530-535.

Zhang, J., Muan, M.A., 1990. Effects of sand burial on seed germination, seedling emergence, survival, and growth of *Agropyron psammophilum*. Can. J. Bot. 68, 304-310.

Table 1. *F*-values for ploidy, seed mass, and population for 12 seedling morphology and growth traits. Temperature main effect is omitted because only one growth chamber was used for each temperature treatment.

Effect	Root initiation	Shoot initiation	Shoot biomass	Root biomass	R:S biomass	Shoot length
Ploidy	12.7***	13.5***	39.9***	36.3***	2.6	20.1***
Seed mass	24.7***	99.0***	10.1**	8.2**	0.3	3.5^{+}
Population	6.9***	28.7***	0.9	2.6^{+}	6.7***	4.6*
Temperature x ploidy	0.0	7.8**	2.9	1.8	4.7*	2.3
Temperature x seed mass	1.7	11.8***	2.8	0.1	1.0	1.4

Effect	Root length	R:S length	SLA	SRL	AGR	RGR
Ploidy	17.8***	0.2	1.4	12.4***	14.3***	1.0
Seed mass	7.2**	8.5**	7.3**	25.2***	2.9^{+}	0.0
Population	4.3**	1.2	0.7	1.4	1.3	1.7
Temperature x ploidy	0.8	0.9	0.2	1.8	0.1	0.0
Temperature x seed mass	15.1***	18.9***	2.1	9.4*	0.4	0.8

$^{+}$, *, **, *** $P < 0.10$, 0.05, 0.01, 0.0001, respectively.

Table 2. Correlation coefficients (r) of seedling traits with seed mass and absolute growth rate (AGR) under low and high temperature regimes.

	Seed mass			AGR		
	Low temp.	High temp.	Across temps.	Low temp.	High temp.	Across temps.
Root initiation	0.48	0.72*	0.60$^+$	0.28	0.70*	0.47
Shoot initiation	0.60$^+$	0.67*	0.60$^+$	0.15	0.57	0.55
Shoot biomass	0.64$^+$	0.84**	0.83**	0.95**	0.86**	0.91**
Root biomass	0.62$^+$	0.91**	0.82**	0.95**	0.78*	0.88**
R: S biomass	0.38	-0.01	0.25	0.59$^+$	-0.31	0.20
Shoot length	0.61$^+$	0.61$^+$	0.67$^+$	0.85**	0.86**	0.88**
Root length	0.58	-0.09	0.19	0.84**	0.24	0.58
R: S length	0.45	-0.54	-0.33	0.65$^+$	-0.45	-0.06
SLA	-0.44	-0.63$^+$	-0.73*	-0.63$^+$	-0.64$^+$	-0.64$^+$
SRL	-0.65$^+$	-0.87*	-0.90**	-0.81**	-0.49	-0.73*
AGR	0.59$^+$	0.65$^+$	0.74*			

$^+$, *, **, *** $P < 0.10, 0.05, 0.01, 0.0001$, respectively.

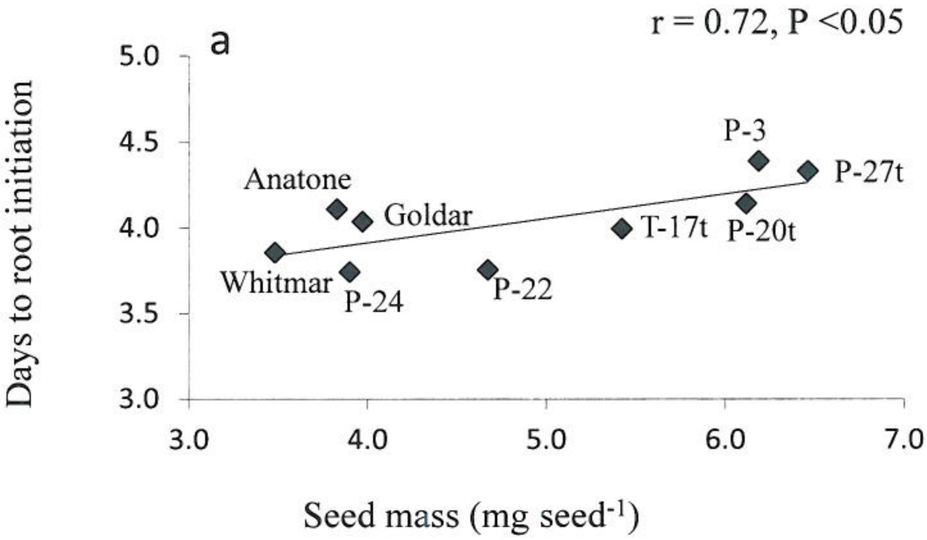
LIST OF FIGURES

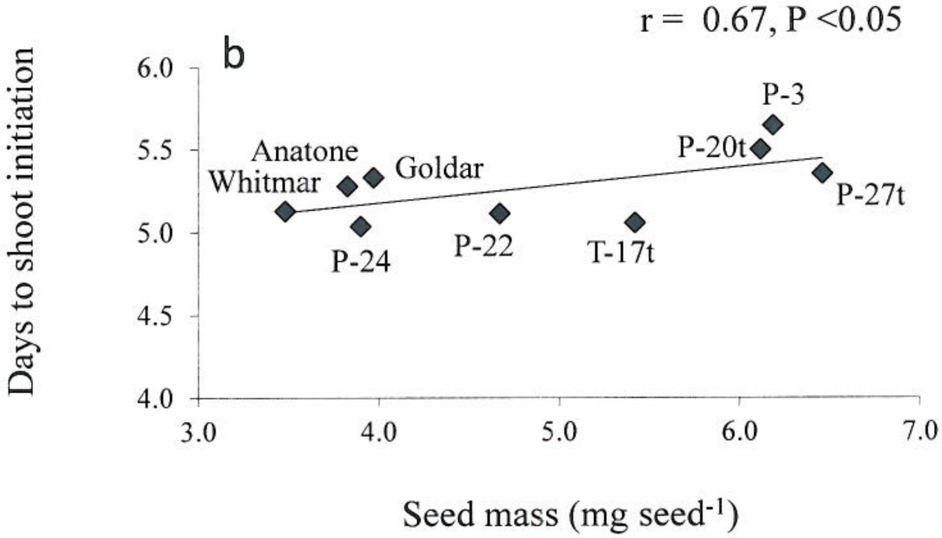
Figure 1. Correlations of seed mass with a) days to root initiation and b) days to shoot initiation across 9 bluebunch wheatgrass populations for the high temperature regime.

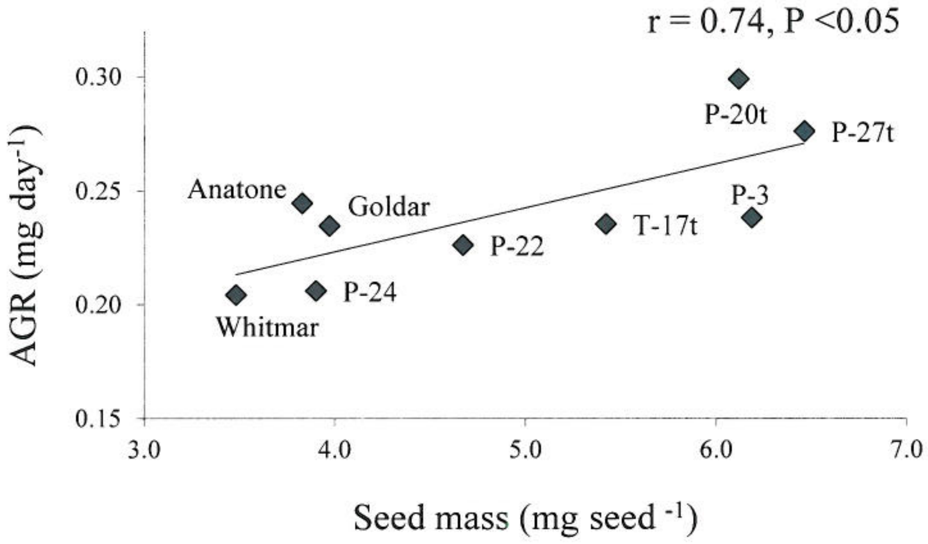
Figure 2. Correlations of seed mass with a) absolute growth rate (AGR), b) specific leaf area (SLA), and c) specific root length (SRL) averaged over 2 temperature regimes across 9 bluebunch wheatgrass populations.

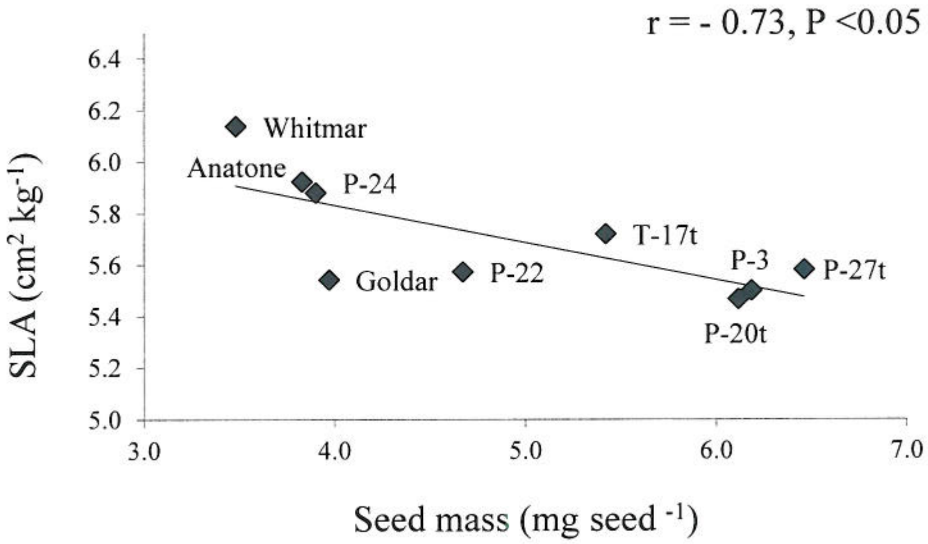
Figure 3. Correlations of absolute growth rate (AGR) with a) specific leaf area (SLA) and b) specific root length (SRL) averaged over 2 temperature regimes across 9 bluebunch wheatgrass populations.

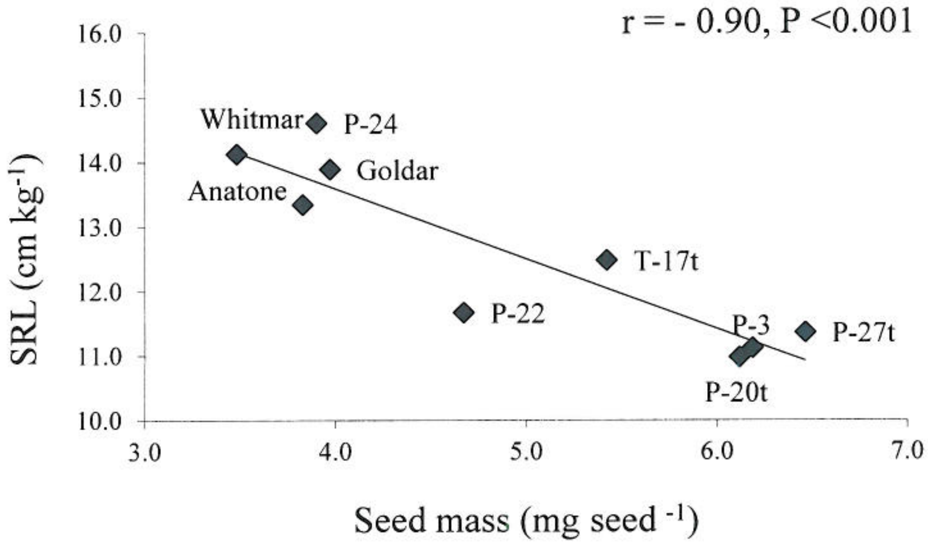
Figure 4. Root-to-shoot length ratio of 9 populations of bluebunch wheatgrass under 2 temperature regimes. Mean separations ($LSD_{0.05}$) are expressed by lower-case and capital letters for low and high temperature regimes, respectively.

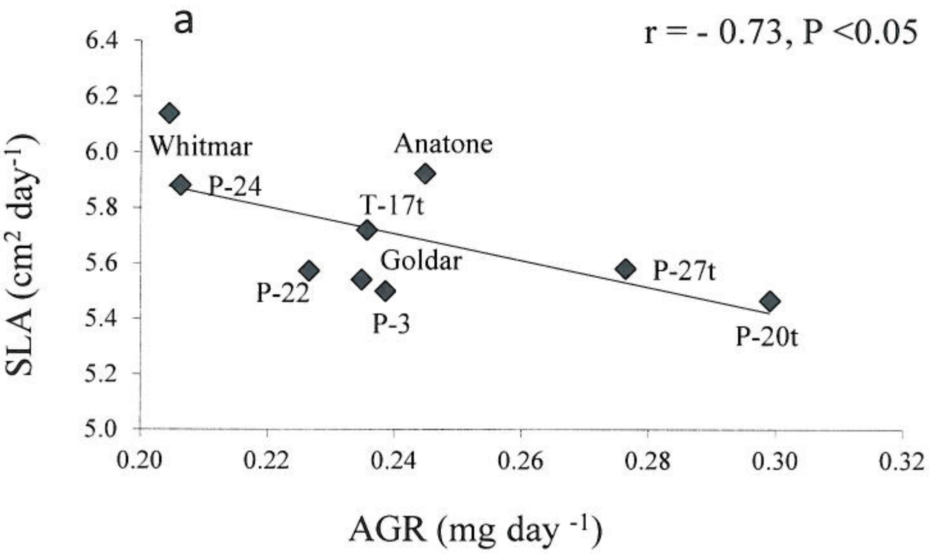

r = 0.72, P <0.05
a
Days to root initiation
5.0
4.5
4.0
3.5
3.0
3.0
4.0
5.0
6.0
7.0
Seed mass (mg seed-1)
Anatone
Goldar
Whitmar
P-24
P-22
T-17t
P-3
P-27t
P-20t

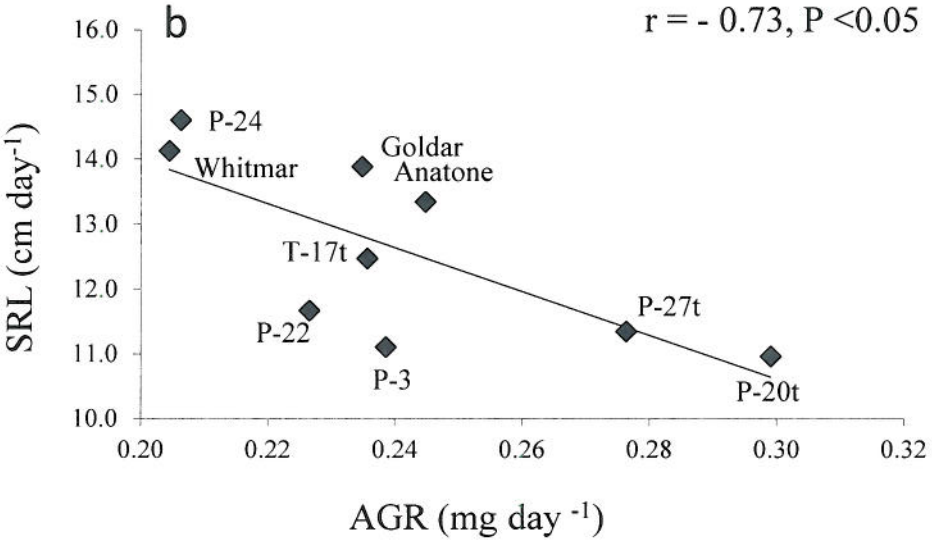

r = 0.67, P <0.05
b
Days to shoot initiation
6.0
5.5
5.0
4.5
4.0
3.0
4.0
5.0
6.0
7.0
Seed mass (mg seed-1)
Anatone
Goldar
Whitmar
P-24
P-22
T-17t
P-3
P-20t
P-27t

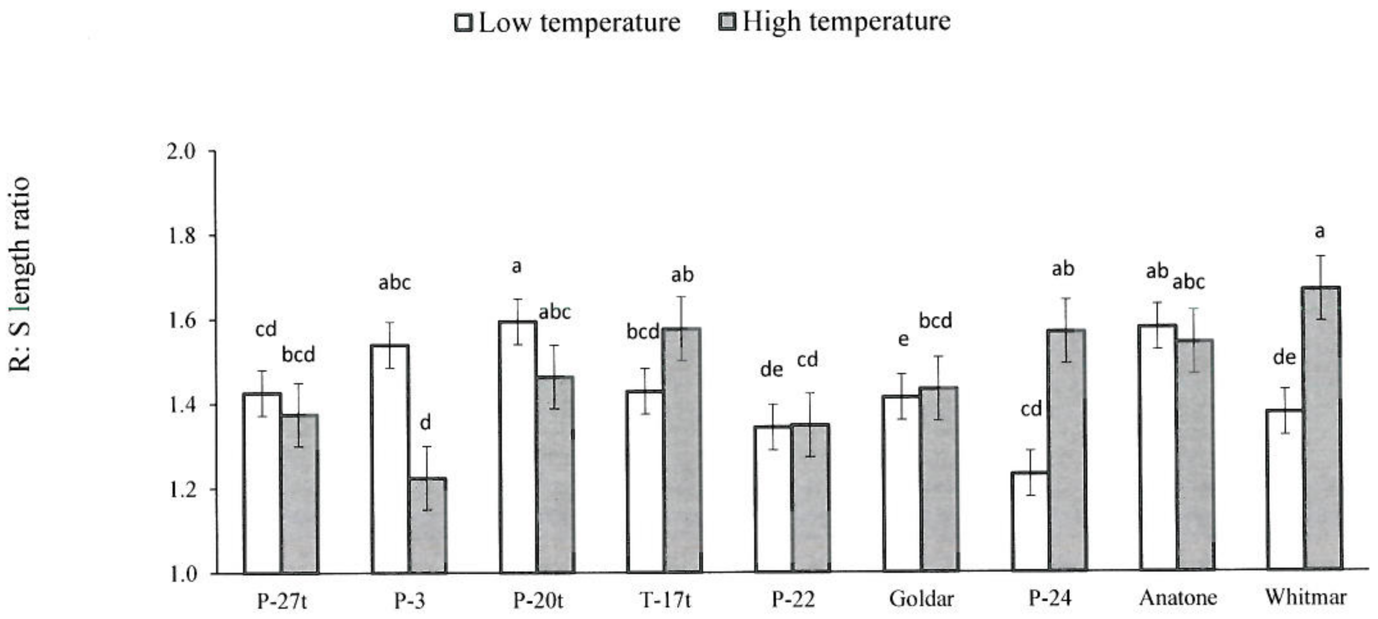

r = 0.74, P <0.05
AGR (mg day^{-1})
0.30
0.25
0.20
0.15
P-20t
P-27t
Anatone
Goldar
P-3
T-17t
P-22
P-24
Whitmar
3.0
4.0
5.0
6.0
7.0
Seed mass (mg seed $^{-1}$)


r = - 0.73, P <0.05
SLA (cm^2 kg^{-1})
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
Whitmar
Anatone
P-24
T-17t
P-3
P-27t
Goldar
P-22
P-20t
3.0
4.0
5.0
6.0
7.0
Seed mass (mg seed $^{-1}$)


r = - 0.90, P <0.001
SRL (cm kg^{-1})
16.0
15.0
14.0
13.0
12.0
11.0
10.0
Whitmar
P-24
Goldar
Anatone
T-17t
P-22
P-3
P-27t
P-20t
3.0
4.0
5.0
6.0
7.0
Seed mass (mg seed $^{-1}$)


a
r = - 0.73, P <0.05
SLA (cm² day⁻¹)
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
Whitmar
Anatone
P-24
T-17t
Goldar
P-27t
P-22
P-3
P-20t
0.20
0.22
0.24
0.26
0.28
0.30
0.32
AGR (mg day ⁻¹)


b
r = - 0.73, P <0.05
SRL (cm day⁻¹)
16.0
15.0
14.0
13.0
12.0
11.0
10.0
P-24
Goldar
Whitmar
Anatone
T-17t
P-27t
P-22
P-3
P-20t
0.20
0.22
0.24
0.26
0.28
0.30
0.32
AGR (mg day ⁻¹)

□ Low temperature
▨ High temperature
R: S length ratio
2.0
1.8
1.6
1.4
1.2
1.0
cd
bcd
abc
d
a
abc
bcd
ab
de
cd
e
bcd
cd
ab
ab
abc
de
a
P-27t
P-3
P-20t
T-17t
P-22
Goldar
P-24
Anatone
Whitmar