Contrasting Mechanisms of Recovery from Defoliation in Two Intermountain-Native Bunchgrasses ☆

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ABSTRACT

Grazing tolerance of dominant native species may determine the fate of rangeland ecosystems, and using native plant populations with good grazing tolerance in restoration seedings may improve ecosystem resilience, especially when domestic herbivores are present. We examined interspecific and intraspecific differences in shoot biomass and defoliation tolerance for two semiarid, perennial cool-season bunchgrasses native to the Intermountain West, USA, Pseudoroegneria spicata and Elymus wawawaiensis, on the basis of four functional traits (specific leaf area [SLA], plant basal area, tiller number, and tiller mass). We applied two treatments, control and boot-defoliation, where the latter included defoliation at the early-reproductive ("boot") stage, the phenological stage most vulnerable to herbivory, while the control treatment did not. We tested two contrasting hypotheses (i.e., that boot-defoliation tolerance is increased through either increases in SLA or through more favorable tiller demography). For shoot biomass, both grasses were less productive under the boot-defoliation treatment than for the control, but E. wawawaiensis displayed higher boot-defoliation tolerance than P. spicata. Interpopulation variation occurred in all four functional traits for P. spicata, but there were no such variation for E. wawawaiensis. The tiller demography hypothesis better explained boot-defoliation tolerance in both species, and neither SLA nor plant basal area was correlated with shoot biomass for either treatment. Of the traits measured, high tiller number served as the primary mechanism for shoot biomass and boot-defoliation tolerance in P. spicata, while tiller number and tiller mass were both important predictors of both shoot biomass and boot-defoliation tolerance.

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Introduction

The native perennial bunchgrasses of the North American Intermountain West are relatively intolerant of grazing, presumably due to weak evolutionary grazing pressure (Mack and Thompson, 1982). Subsequent to widespread settlement in this region in the 1840s, uncontrolled grazing by livestock exerted a dominant influence on arid and semiarid ecosystems by destabilizing native-plant communities (Fleischner, 1994) and increasing the abundance and impacts of invasive plants (Fleischner, 1994), thereby reducing livestock productivity (DiTomaso, 2000). The lack of grazing tolerance in Intermountain-native bunchgrasses was one factor that motivated widespread seeding of *Agropyron desertorum* (Fisch. ex Link) Schult., a grazing-tolerant, exotic perennial bunchgrass (Caldwell et al., 1981; Monsen et al., 2004; Richards and Caldwell, 1985). However, because

native plants are often preferred by restoration practitioners and livestock grazing continues to be widespread on these rangelands, the development of grazing-tolerant native plant materials is a worthwhile goal.

Plant functional traits are legacies of historical selective pressures, and recent ecological studies provide a framework for evaluating plant responses to various land-management treatments on the basis of functional traits (Adler et al., 2004, 2005; Diaz et al., 1999, 2001). Species with high relative growth rates also have high specific leaf area (leaf area per unit biomass; SLA) and are known to be more productive. Hence in these species, SLA serves as the best single indicator for the underlying mechanisms of plant growth (Garnier et al., 2001; Poorter and Garnier, 2007; Ray-Mukherjee et al., 2011), an attribute that might also confer higher potential for recovery following defoliation (Poorter and Garnier, 2007; Poorter et al., 2009; Reich et al., 1999, 2003).

Studies of herbaceous species have shown that defoliation may increase SLA (Guo et al., 2008; Rotundo and Aguiar, 2008; but see Thorne and Frank, 2009). Diaz et al. (2001) and showed that although SLA may not be the best predictor of grazing response overall, grazing tolerance is achieved through high SLA in species with a short-term evolutionary grazing history. A contrasting hypothesis, mediated through tiller demography, suggests that herbivory in grasses may increase tiller number per unit of plant basal area, initiating active tillering in the

periphery of fragmented plants (Butler and Briske, 1988; Caldwell et al., 1981). However, less is known about the interrelationships among grazing-response traits, such as biomass production, SLA, tiller number, tiller mass, and plant basal area, in the presence and absence of defoliation.

Traits related to defoliation tolerance involve confounded interactions between genotypes and their historical selection pressures (Adler et al., 2004; Mack and Thompson, 1982; Tiffin and Rausher, 1999); climatic conditions (Adler et al., 2004; Milchunas et al., 1988); and intensity, frequency, and timing of defoliation (Del-Val and Crawley, 2005). Higher intensity and frequency of defoliation affect the plant's ability to recover (Maschinski and Whitham, 1989; McNaughton, 1983) and to persist in its historical range (Sheley et al., 2008). In addition to defoliation intensity, timing of defoliation strongly influences a plant's ability to recover (Maschinski and Whitham, 1989; McNaughton, 1985). In semiarid cool-season bunchgrasses, defoliation at the early-reproductive, or "boot," stage (i.e., when the culm is swollen with the inflorescence before its extension above the flag leaf [Wilson et al., 1966]), is more detrimental than defoliation at either earlier (vegetative) or later (late-reproductive) stages (Blaisdell and Pechanec, 1949; Cook and Stoddart, 1963; Ganskopp, 1988; Jones and Nielson, 1997; Sheley et al., 1997; Wilson et al., 1966). Defoliating plants at a common phenological stage will thus impose stronger selection pressure and will permit a more reliable physiological evaluation of defoliation response.

Bunchgrasses native to North America's Intermountain West possess an array of morphological and physiological traits that may contribute to their survival and success under varying grazing pressures. The presence of inter- and intra-specific variation for these traits (Violle et al., 2012) may assist selection of defoliation-tolerant plant materials for restoration purposes. To this end, identification of easily measured plant functional traits that could serve as indicators of grazing response would be desirable (Diaz et al., 2001). *Pseudoroegneria spicata* (Pursh.) Á. Löve and *Elymus wawawaiensis* Carlson and Barkworth (1997) are two native perennial bunchgrasses of this region that are valued for both grazing and restoration applications. In order to understand inter- and intra-specific variation in grazing response for these two grasses, we applied defoliation at the boot stage for two consecutive years. Our objectives were, 1) to compare species and populations for boot-defoliation tolerance, SLA, plant basal area, tiller number, and tiller mass 2) to assess the impact of boot-defoliation on SLA, plant basal area, tiller number and tiller mass, and 3) to quantify the contributions of these functional traits to shoot biomass production and boot-defoliation tolerance, thereby testing the relative merit of the SLA and the tiller demographic hypotheses of defoliation tolerance.

Materials and Methods

Study Species

Pseudoroegneria is a widespread native, C_3 perennial bunchgrass that was once dominant across the Intermountain West (Mack and Thompson, 1982). Consequently, this grass is in demand for rangeland restoration applications (Monsen et al., 2004). Poor grazing tolerance, coupled with high palatability, is believed to be responsible for the decline of this species (Adler et al., 2004; Caldwell et al., 1981; Jones and Nielson, 1997; Mueggler, 1972; Richards and Caldwell, 1985; Richards et al., 1988). Newly generated photosynthetic area is important for *P. spicata*'s recovery from defoliation (Richards and Caldwell, 1985), but recovery is compromised by prolonged investments in root growth at the expense of shoot growth, even after severe defoliation (Caldwell et al., 1981).

The natural distribution of *E. wawawaiensis* is much more restricted than that of *P. spicata,* being confined to eastern Washington and Oregon and the panhandle of Idaho in the northern Intermountain West. But while *E. wawawaiensis* is native to the northern Intermountain

West, most of its restoration applications relate to wildfire rehabilitation to the south of its natural distribution (in the central Intermountain West), where *P. spicata* is native. *Elymus wawawaiensis* (released population "Secar") displays superior defoliation tolerance, seedling establishment, and drought tolerance to that of most commercially available *P. spicata* plant material (Jones and Nielson, 1997; Monsen et al., 2004; Morrison and Kelley, 1981; Ogle, 2002). Consequently, *E. wawawaiensis* has become widely used as a surrogate for *P. spicata* in the central Intermountain West (Jones 2003).

Study Populations

For this study, we used two released *P. spicata* populations ("Goldar" and Anatone Germplasm), three experimental *P. spicata* populations (P-22, P-26, and P-27t), two released *E. wawawaiensis* populations ("Secar" and "Discovery"), and one experimental *E. wawawaiensis* population (E-46). These populations were included because they are currently commercially available for restoration applications in the region (termed *released*) or are being considered for such use (termed *experimental*).

Of the experimental *P. spicata* populations, P-22 was developed from P-1, a population of unknown origin, by three cycles of selection for general vigor and spike production and a fourth for recovery from defoliation. P-26 was developed from the released P-7 germplasm, a genetically diverse polycross, by three cycles of selection for general vigor and spike production (with no emphasis on defoliation tolerance). P-27t, the lone tetraploid *P. spicata* population in the study, was developed by two cycles of selection for seedling vigor and spike production from P-8. P-8, in turn, was experimentally induced by colchicine treatment from the diploid P-3, a polycross of several populations collected along the Grande Ronde River in northeastern Oregon. E-46 *E. wawawaiensis* was developed from Discovery *E. wawawaiensis* by one cycle of selection for defoliation tolerance and another cycle for general vigor and spike production.

Experimental Procedure

Seedlings were transplanted into a 3:1 volumetric mixture of sand and peat moss in opaque plastic tubes (20-cm long and 5-cm wide). Plants were reared in a greenhouse in Logan, Utah and were trimmed once to a 15-cm height before transplanting to a field site (lat 41°39′25′′N, long 111°48′51′′W; 1 437 m asl; Ricks gravelly loam [coarse-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Calcic Haploxerolls]) near Millville, Utah. This site is typical of a semiarid sagebrush semidesert site in the western foothills of the Wasatch Mountains, but it had been cleared of preexisting vegetation. Long-term average precipitation of the field site was 388 mm, and the site experienced 315 mm rainfall in 2007, 369 mm in 2008, and 469 mm in 2009 (Table S1, available online at [URL]). Similarly, the long-term data showed that temperature ranged approximately from -10°C to 35°C, and our study site experienced minimum monthly mean temperature of -14.2°C (2007), -14.9°C (2008), and -14.2°C (2009) and a maximum monthly mean temperature of 35.4°C (2007), 33.5°C (2008), and 31.4°C (2009) during the study period (Table S2, available online at [URL]).

The experimental planting was established on 5 June 2006. Each of 15 replicates consisted of eight randomized whole plots (see next paragraph) corresponding to the eight populations, with each whole plot consisting of two plants of its assigned population planted side-by-side. The 16 plants of each replicate were spaced 1-m apart in both directions (see Fig. S1). To provide similar levels of competition to all experimental plants, the entire experimental area was surrounded by an equidistant (1-m) single-row border of Goldar *P. spicata*. All plants were harvested at 10 cm on 29 October 2006 (Table S3B; available online at [URL]) before defoliation treatments were imposed beginning in 2007. Because both species are cross-pollinated, this design provided 75 (15 replications × 5 populations) distinct genotypes of *P. spicata* and

45 (15 × 3) distinct genotypes of *E. wawawaiensis* to search for possible mechanisms of boot-defoliation tolerance.

We employed a split-plot design with population as the whole-plot factor and defoliation treatment as the split-plot factor. In 2007, we randomly selected one of the two individuals of each population within each whole plot for the boot-defoliation treatment, while the remaining individual was left undefoliated at the critical boot stage (control). Individual plants in the boot-defoliation treatment were initially defoliated when they reached the boot stage (see Table S3A, Fig. S2). A reproductive tiller is in the boot stage when the culm is swollen with its inflorescence, and an individual plant was declared to be in the boot stage when inflorescences had begun to emerge from 10 tillers. The average initial defoliation date was 26.0 ± 0.5 days earlier in 2007 than 2008 ($P < 0.0001$) (Fig. S2; available online at [URL]). On average, initial defoliations were 2.4 ± 0.5 days earlier for *E. wawawaiensis* populations than *P. spicata* populations ($F_{1,231} = 16.68$, $P < 0.0001$). Subsequent defoliations (three in 2007, but only two in 2008 because of drought) were made for the boot-defoliation treatment at 4-week intervals (see Table S3B). Harvest for the control treatment was delayed until 13 August in 2007 and 12 August in 2008 because by this time reproduction had ended and the plants could be defoliated without detriment. For the control treatment, all plants were defoliated on a common date. An end-of-year defoliation was applied to both treatments on 10 October 2007 and 20 October 2008, and a final biomass determination was made for both treatments on 19 June 2009. Throughout the experiment, defoliation was applied at a 10-cm height.

Additional Data Collection

On 3-4 May 2008, subsamples of five fully open, mature leaves of similar-age classes from each plant were passed through an LI-3100C leaf-area meter (LI-COR, Inc. Lincoln, NE), after which leaves were dried (60°C for 2 days) and weighed to determine SLA (Cornelissen et al., 2003). We also counted tillers on defoliated and control plants shortly before the initial 2008 defoliation of the boot-defoliation treatment. On 3-4 October 2008 we again determined SLA, but for this measurement, leaf area was determined using WinRHIZO Pro Version 2005b (Reagent Instrument, Inc., Québec City, Canada). On these same dates in October 2008, we determined circumference of individual plants with a tape measure. We then calculated plant basal area (πr^2) from circumference ($2\pi r$), assuming it to be circular.

Data Analysis

In both 2008 and 2009, we estimated percentage boot-defoliation tolerance as the ratio of the annual total of shoot biomass (dry weight) of each boot-defoliated plant to its neighboring control plant × 100 (Jones and Nielson, 1997). This gave us separate boot-defoliation tolerance measurements after 1 year (2007) of boot-defoliation (calculated from the sum of all defoliations in 2008) and after 2 years (2007, 2008) of boot-defoliation (calculated from the single 2009 harvest). Tiller number comparisons were made in two ways. First, we compared tiller number between the treatments after 1 year of boot-defoliation (in May 2008), and second, we determined tiller number for the boot-defoliation treatment shortly before each defoliation (see supplemental text on page 4 and Table S4 (available online at [URL]). Similarly, tiller mass was compared between the treatments after 1 year of boot-defoliation (in May 2008). Data from the first comparison of tiller number and tiller mass were analyzed as a general linear analysis of variance (ANOVA), while we performed repeated-measures ANOVA for the second comparison. Data were analyzed using SAS 9.2 (SAS Institute, Cary, NC, 2008).

Species, populations, boot-defoliation treatments, and time periods were considered to be fixed factors, while replications were considered to be random. Data for shoot biomass, boot-defoliation tolerance, SLA (Table 1), and defoliated-tiller regrowth (see Table S4) were analyzed with repeated measures in PROC MIXED using an autoregressive model with heterogeneous variance [arh (1)] under the assumption that variance of the measured traits increased over years. The determination of plant basal area, tiller number, and tiller mass in 2008 were also analyzed using PROC MIXED, but these data were not repeated (Table 2). Data were transformed when normality assumptions were not met. A square-root transformation was used for shoot biomass, and a log transformation was used for SLA, defoliation tolerance, and tiller mass. We compared species and years using CONTRAST statements. We separated back-transformed least-squares means with an $LSD_{0.05}$. However, standard errors were calculated from the nontransformed data set.

To determine if any traits we measured explained variation in boot-defoliation tolerance, we conducted a multiple regression with tiller number, tiller mass, SLA, and plant basal area as potential predictor (independent) variables. As these variables were correlated with each other to various extents, we used commonality analysis (Ray-Mukherjee et al., 2014) to characterize their contribution to shoot biomass and boot-defoliation tolerance. Commonality analysis decomposes variance into unique and common (shared among independent

Table 1
Repeated measures analysis of variance (*F* values) for shoot biomass, boot-defoliation tolerance, and specific leaf area (SLA) of 5 *Pseudoroegneria spicata* (PSSP) and 3 *Elymus wawawaiensis* (ELWA) populations in response to boot-defoliation treatment. Boot-defoliation tolerance was calculated from total annual biomass for 2008 and 2009 separately.

	No. df	Shoot biomass (g) 2007, 2008, 2009	No. df	Boot-defoliation tolerance (%) 2008, 2009	SLA (m² kg⁻¹) May, Oct. 2008
Year	2	106.83***	1	11.23**	681.231***
Species	1	5.86*	1	12.75**	86.82***
Population (species)	6	1.21	6	0.39	5.47***
PSSP populations	*4*	*1.22*	*4*	*0.31*	*6.94****
ELWA populations	*2*	*1.00*	*2*	*0.35*	*1.06*
Year* species	1	17.22***	1	4.03*	0.00
Year* population (species)	6	3.35***	6	2.12⁺	3.70**
Treatment	1	797.92***	1	-	4.73*
Treatment* year	2	191.07***	1	-	1.54
Treatment* species	1	15.61***	1	-	1.85
Treatment* population (species)	6	1.12	6	-	0.26
Treatment PSSP populations*	*4*	*1.25*	*4*	*-*	*2.05⁺*
Treatment ELWA populations*	*2*	*0.91*	*2*	*-*	*0.67*
Treatment* year* species	1	2.22	1	-	2.15
Treatment* year* population(species)	6	0.74	6	-	0.89

*** $P < 0.0001$.
** $P < 0.01$.
* $P < 0.05$.
⁺ $P < 0.10$.

Table 2
Analysis of variance (F values) for plant basal area (August 2008), tiller number (May 2008), and tiller mass (May 2008) of 5 *Pseudoroegneria spicata* (PSSP) and 3 *Elymus wawawaiensis* (ELWA) populations in response to defoliation treatments.

	No. df	Plant basal area (cm^2) August 2008	Tiller number (per plant) May 2008	Tiller mass (g) May 2008
Species	1	10.87**	0.34	7.59**
Populations (species)	6	2.24**	8.28***	10.83***
PSSP populations	4	*2.98**￼*	*11.41***￼*	*14.38***￼*
ELWA populations	2	*0.26*	*1.58*	*1.54*
Treatment	1	151.04***	70.83***	509.86***
Treatment* species	1	6.30**	3.99*	0.60
Treatment* population (species)	6	1.68	1.38	3.46**
Treatment PSSP populations*	4	*1.49*	*1.33*	*4.46**￼*
Treatment ELWA populations*	2	*2.33*	*0.86*	*0.89$^+$*

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

variables) effects and identifies the magnitude and location of multicollinearity in the model. Using the "yhat" package (Nimon et al., 2013) in R (R development Core Team, 2013), we computed path coefficients (also referred to as standardized partial regression coefficients) (β), structure coefficients (r_s), unique coefficients (U), commonality coefficients (C) (Table 3), and the total contribution of each predictor variable and each set of multiple variables (Table S5; available online at [URL]) in determining shoot biomass and boot-defoliation tolerance. Structure coefficients, unlike path coefficients, are independent of multicollinearity, and the square of an independent variable's structure coefficient (r_s^2) quantifies the proportion of the model variance explained by that variable (Ray-Mukherjee et al., 2014). Unique and commonality coefficients quantify the unique contributions of each variable and the common contributions of sets of variables, respectively, to the overall model R^2 (Ray-Mukherjee et al., 2014). All values used in these calculations were corrected for replication effects.

Results

Over the course of the study, the total average boot-defoliated shoot biomass was reduced by 66.1% (see Table 1, Fig. 1). In the first year of defoliation (2007), boot-defoliation reduced shoot biomass 22.9%, but this effect increased to 85.8% in 2008 and 79.1% in 2009. For the control, the two species were similar for shoot biomass in 2007. However, in 2008 for the control treatment, *P. spicata* populations were 18.8% more productive than those of *E. wawawaiensis* in 2008, while in 2009 *E. wawawaiensis* populations were 18.9% more

productive than *P. spicata* populations (see Fig. 1). Alternatively, for the boot-defoliation treatment, shoot biomass was similar for the two species in 2007 and 2008, while in 2009 *E. wawawaiensis* was 132% more productive than *P. spicata,* indicating a tendency for better recovery from boot-defoliation in *E. wawawaiensis* (see Fig. 1). The substantially higher 2009 shoot biomass of *E. wawawaiensis* under boot-defoliation, relative to *P. spicata,* was especially apparent for Discovery, which displayed a 127% increase from 2008–2009 (see Fig. 2b). Increases were also evident in Secar (53%) and E-46 (62%) during the same time span (see Fig. 2b).

In 2008, the second year of boot-defoliation, boot-defoliation tolerance averaged 13.7 ± 2.6% across the eight populations, while in 2009 average boot-defoliation tolerance was somewhat higher (18.8 ± 2.9%). Averaged over the 2 years (2008 and 2009) of measurements, boot-defoliation tolerance of *E. wawawaiensis* was 75.4% (21.2 ± 3.0%) greater than for *P. spicata* (12.1 ± 2.4%) (see Table 1). Compared with that in 2008, boot-defoliation tolerance in 2009 was 65.0% greater for *E. wawawaiensis* populations (27.3 ± 3.2 vs. 16.5 ± 2.8%) and only 13.9% greater for *P. spicata* populations (12.9 ± 2.5 vs. 11.3 ± 2.4%), giving rise to a year × species interaction (see Table 1).

Averaged over the two 2008 measurements, the SLA of *E. wawawaiensis* was 25.6% greater than that of *P. spicata* (see Table 1, Fig. 3). The three *E. wawawaiensis* populations were similar for SLA, but P-27t, the sole tetraploid *P. spicata* population, displayed lower SLA than diploids Anatone or Goldar. Averaged across populations, SLA was 78.7% greater in October 2008 than in May 2008. Among *P. spicata* populations, Anatone showed the greatest increase in SLA

Table 3
Beta coefficients (β), squared structure coefficients (r_s^2), unique coefficients (U), and commonality coefficients (C) of 4 independent variables that may explain the variance of 2 dependent variables, boot-defoliated shoot biomass, and boot-defoliation tolerance.

	Shoot biomass				Boot-defoliation tolerance			
	β	r_s^2[1]	U[2]	C[3]	β	r_s^2	U	C
Pseudoroegneria spicata								
SLA	-0.220	0.014	0.000	0.012	-0.122	0.002	0.013	-0.013
Basal area	0.017	0.058	0.000	0.048	-0.140	0.002	0.018	-0.018
Tiller number	1.054***	0.923	0.688	0.068	0.541***	0.857	0.182	0.034
Tiller mass	0.306***	0.127	0.055	0.050	0.024	0.213	0.000	0.053
Elymus wawawaiensis								
SLA	-0.081	0.066	0.006	0.050	-0.165	0.184	0.026	0.050
Basal area	-0.089	0.068	0.007	0.052	-0.001	0.110	0.000	0.046
Tiller number	0.733***	0.350	0.447	-0.145	0.435**	0.276	0.157	-0.043
Tiller mass	0.759***	0.429	0.516	-0.145	0.502***	0.466	0.226	-0.034

SLA indicates specific leaf area.
*** $P < 0.0001$.
** $P < 0.01$.
* $P < 0.05$.
$^+$ $P < 0.10$.
[1] Proportion of the variation in the dependent variable explained by an independent variable (sum of unique and common contributions).
[2] Amount of explained variation in the dependent variable that is the unique to an independent variable.
[3] The total *common* contributions of an independent variable in all possible subsets of regression involving that independent variable.
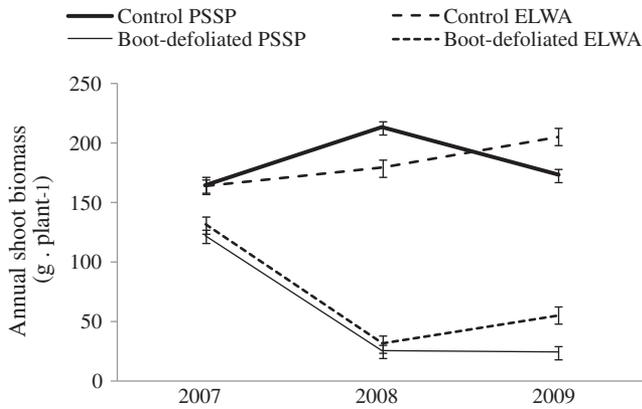
Fig. 1. Mean annual shoot biomass for control and boot-defoliation (applied in 2007 and 2008) treatments across five *Pseudoroegneria spicata* (PSSP) and three *Elymus wawawaiensis* (ELWA) populations.
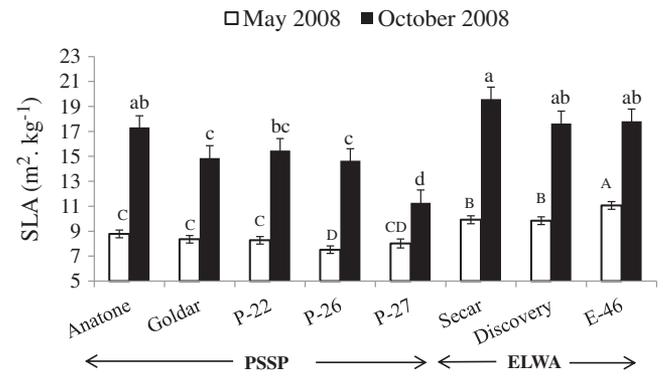

Fig. 3. Means and standard errors for specific leaf area (SLA) in May and October 2008 for five *Pseudoroegneria spicata* (PSSP) and three *Elymus wawawaiensis* (ELWA) populations. Different letters represent significant ($P < 0.05$) differences among populations in May (upper case) and October (lower case).

from May to October, while P-27t displayed the smallest increase (see Fig. 3). Among *E. wawawaiensis* populations, Secar displayed the greatest increase in SLA from May to October 2008, while E-46 exhibited the smallest increase (see Fig. 3). In our study, boot-defoliation significantly reduced SLA, but only by 5% on average (see Table 2).

The two species had similar plant basal area for the control treatment, but boot-defoliation reduced plant basal area 35.1% (340.9 ± 16.3 vs. 221.1 ± 16.4 cm^2) for *E. wawawaiensis* and 50.2% ($329.6 \pm$

13.6 vs. 163.9 ± 13.6) for *P. spicata*, resulting in a treatment x species interaction (see Table 2). However, populations within species did not interact with treatment for plant basal area (see Table 2). Averaged across treatments, *P. spicata* populations P-22 (267.0 ± 19.0 cm^2), Goldar (286.4 ± 19.2), and Anatone (240.0 ± 19.0) had greater plant basal area than P-27t (210.3 ± 19.5) and P-26 (210.3 ± 19.2), but we detected no differences among *E. wawawaiensis* populations for this trait (see Table 2).
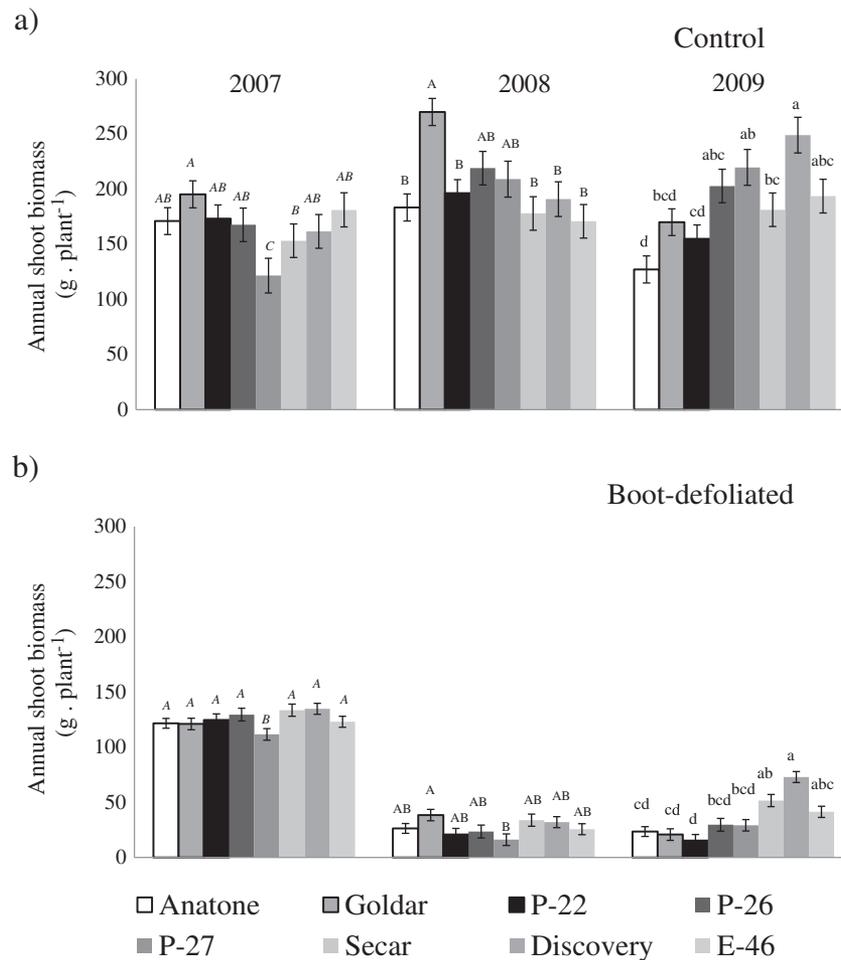

Fig. 2. Means and standard errors for annual shoot biomass of five *Pseudoroegneria spicata* (Anatone, Goldar, P-22, P-26, P-27t) and three *Elymus wawawaiensis* (Secar, Discovery, E-46) populations from 2007–2009 for (a) control and (b) boot-defoliated treatments. Different letters represent significant ($P < 0.05$) differences among populations within a year.

For tiller number, we found differences among *P. spicata* populations, with Goldar displaying the most tillers (249.0 ± 20.0) and P-27t the fewest (92.0 ± 21.0) (see Table 2). On the other hand, *E. wawawaiensis* populations did not differ for tiller number (166.61.0 ± 19.6) (see Table 2). After 1 year of boot-defoliation (May 2008), *E. wawawaiensis*

tiller number was 40.2% lower than that of the control (205.7 ± 17.2 vs. 123.1 ± 13.8), while tiller number of *P. spicata* was more seriously affected, decreasing by 57.5% (228.2 ± 14.2 vs. 97.1 ± 16.5) (see Table 2).

On average, *P. spicata* had13.4% more tiller mass than *E. wawawaiensis*, irrespective of boot-defoliation. Among *P. spicata* populations, P-27t
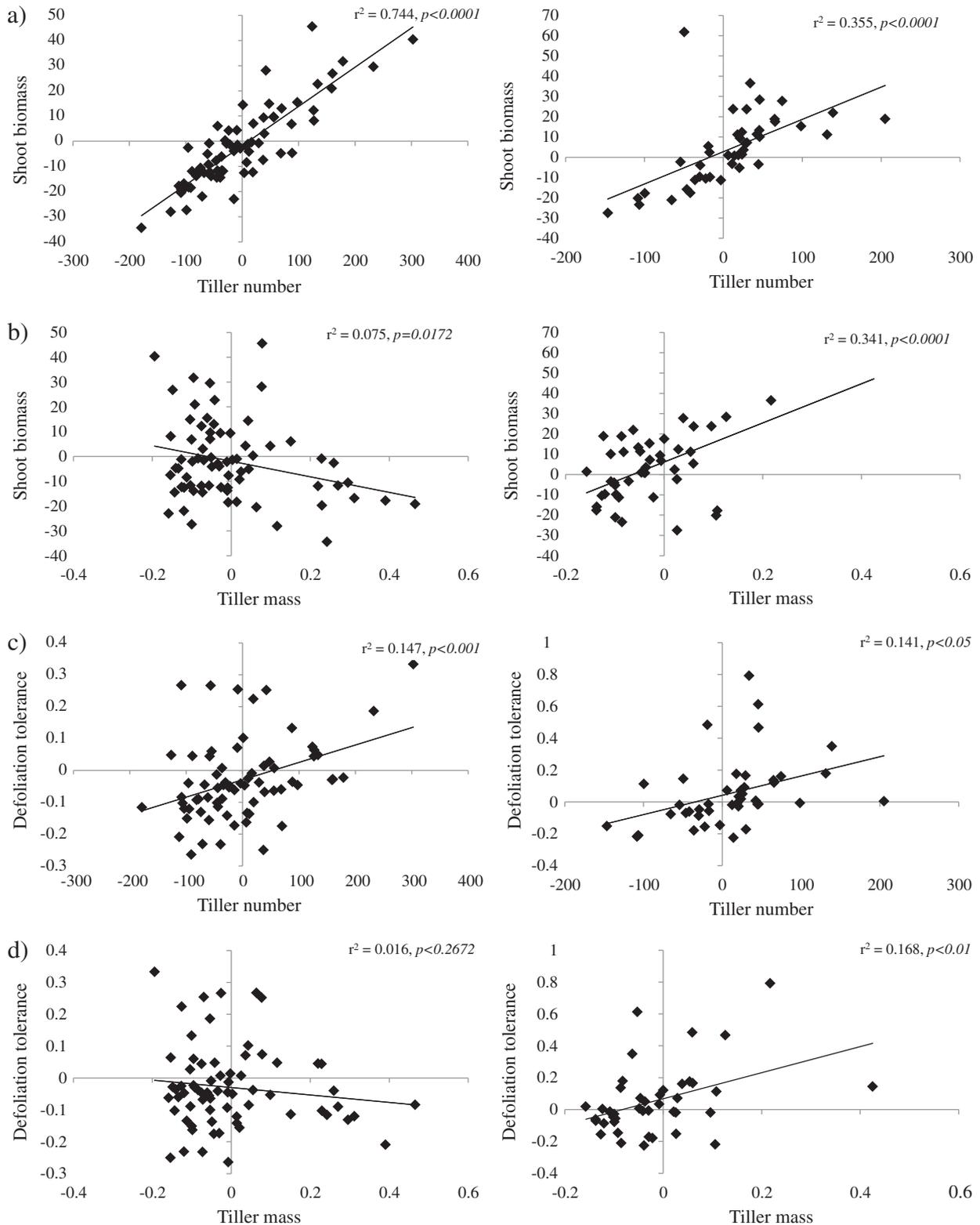

Fig. 4. Correlations for the boot-defoliation treatment between (a) tiller number and biomass, (b) tiller mass and biomass, (c) tiller number and defoliation tolerance, and (d) tiller mass and defoliation tolerance for *Pseudoroegneria spicata* (left) and *Elymus wawawaiensis* (right).
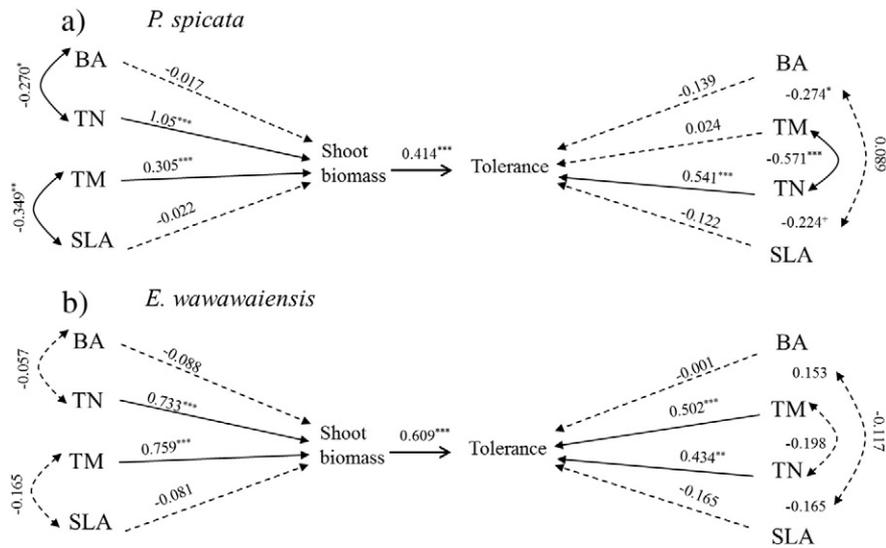
Fig. 5. Path model showing how variation in the basal area (BA), SLA, tiller number (TN), and tiller mass (TM) affects shoot biomass and defoliation tolerance differently in *P. spicata* (BBWG) and *E. wawawaiensis* (SRWG) across control and defoliation treatment. Each path effect show the path coefficients (standardized partial regression coefficient) and the significance level of the path. Solid lines represent significant relationships and dashed lines represent non-significant relationship.

(1.215 ± 0.09), P-22 (1.202 ± 0.10), and P-26 (0.999 ± 0.09) displayed greater tiller mass than other populations under the control treatment, while P-27t (0.459 ± 0.10) displayed greatest tiller mass under boot-defoliation. *E. wawawaiensis* populations, on the other hand, did not differ in tiller mass (see Table 2).

For *P. spicata,* tiller number stood out as the strongest determinant of shoot biomass, accounting for 92.3% ($r_s^2 = 0.923$) of the model R^2 (in this case, $R^2 = 0.819$, $R^2_{adj} = 0.807$), followed by tiller mass that significantly explained 12.7% ($r_s^2 = 0.127$) of the variation in R^2 (see Table 3, Fig. 4a and b, Fig. 5; Table S5). In this case, the contributions of SLA and plant basal area to shoot biomass were only indirect, occurring via common contributions with other predictors (see U vs. C in Table 3). For shoot biomass of *E. wawawaiensis*, tiller number ($r_s^2 = 0.350$) and tiller mass ($r_s^2 = 0.429$) contributed 35.0 % and 42.9%, respectively, of the explained variation in the model ($R^2 = 0.863$, $R^2_{adj} = 0.848$) (see Table 3, Fig. 4a and b, Fig. 5; Table S5). Similar to *P. spicata*, for *E. wawawaiensis* the contributions of SLA and basal area to the model were negligible, again mostly through common contributions (U vs. C in Table 3). It is also important to note that, in the case of *P. spicata*, tiller number and tiller mass jointly contributed only 2.4% of the variation in shoot biomass (commonality coefficient = 0.019, Table S5), while in the case of *E. wawawaiensis*, the joint contribution was 23.6% and negative (commonality coefficient = -0.203, Table S5). This highly negative joint contribution is due to negative multicollinearity between these two traits (Table S6; available online at [URL]).

Results for boot-defoliation tolerance were similar to those for shoot biomass for both species. For *P. spicata*, tiller number ($r_s^2 = 0.857$) rather than tiller mass ($r_s^2 = 0.213$) stood out as the greatest determinant (85.7% of R^2) of boot-defoliation tolerance ($R^2 = 0.251$, $R^2_{adj} = 0.201$) (see Table 3, Fig. 4c and d; Table S5). In this species, the contributions of SLA ($r_s^2 = 0.002$), basal area ($r_s^2 = 0.002$), and tiller mass (U = 0.000) to boot-defoliation tolerance were negligible (see Table 3). However, in the case of *E. wawawaiensis*, tiller mass ($r_s^2 = 0.466$) was the greatest driver of boot-defoliation tolerance ($R^2 = 0.251$, $R^2_{adj} = 0.201$), explaining 46.6% of the variation in the model, followed by tiller number ($r_s^2 = 0.276$), which explained 27.6% of the variation in boot-defoliation tolerance (see Table 3; Fig. 4c and d; and Table S5). Similar to *P. spicata,* SLA and basal area did not contribute to the boot-defoliation tolerance of *E. wawawaiensis* (see Table 3). Similar to shoot

biomass, tiller number and tiller mass jointly contributed 27.2% (commonality coefficient = 0.068, see Table S5) to the variation in boot-defoliation tolerance for *P. spicata*, while for *E. wawawaiensis* the same was -19.2% (commonality coefficient = -0.079), suggesting confounding effects between the two variables.

Discussion

Intense defoliation may stimulate overcompensation in humid ecosystems (Ruiz et al., 2008; van der Heyden and Stock, 1996), but limited moisture availability in arid and semiarid environments often impedes regrowth, resulting in undercompensation (Belsky, 1986; Ruiz et al., 2008). In our study, both species produced less total biomass under boot-defoliation than for the control, although *E. wawawaiensis* was more boot-defoliation tolerant than *P. spicata*. Although we found a significant difference between species for boot-defoliation tolerance, populations within each species did not differ significantly for this trait.

Most studies, except Blaisdell and Pechanec (1949), Cook and Stoddart (1963), Wilson et al. (1966), Ganskopp (1988), Sheley et al. (1997), and Jones and Nielson (1997), have imposed defoliation on a single common date regardless of phenological stage, compromising the relevance of phenology to the defoliation treatment. Although such an experimental approach simulates the management practice of initiating grazing of multiple species on a single calendar date (in a common paddock), individual species, populations, and plants often vary for phenological stage at a common date. In contrast, by applying defoliation at a common phenological stage, as done in this study, we were able to more appropriately and accurately measure the impact of defoliation.

Plant Traits and Determinants of Defoliation Tolerance

Many researchers have examined the role of SLA as a mechanism for defoliation tolerance. Wilson et al. (1999) proposed that SLA is an important trait for resource capture. High-SLA leaves are more productive and reflect higher expected carbon return on previously invested carbon (Rotundo and Aguiar, 2008; van der Meijden et al., 1988; Wilson et al., 1999). High SLA has been associated with both grazing-avoidance and grazing-tolerance strategies. For example, defoliation of a given leaf

area will remove less biomass from a high SLA (area/biomass) species compared with a low SLA species, suggesting grazing avoidance (Rotundo and Aguiar, 2008). On the other hand, high SLA translates into higher relative growth rate and faster leaf-area replacement, indicating grazing tolerance (Diaz et al., 2001; McNaughton, 1984). Nevertheless, leaves of high-SLA species are shorter-lived and more vulnerable to herbivory (Coley et al., 1985; Grime, 2001; Wilson et al., 1999). Diaz et al. (2001) compared sets of species (grasses and dicot herbaceous species) and different grazing-response strategies (grazing increasers or grazing-resistant species and grazing decreaser or grazing-sensitive species) from Israel and Argentina, but they found no relationship between grazing response and SLA. Instead, their study showed that plant height and then leaf mass were better determinants of grazing response.

Like Diaz et al. (2001), our data did not suggest any contribution by SLA to boot-defoliation tolerance. We anticipated that boot-defoliation would increase SLA (Guo et al., 2008), but we found instead a 5% reduction that was consistent across species. Therefore our finding was consistent with greenhouse studies on grasses for which differences in SLA between control and defoliated plants were small (Rotundo and Aguiar, 2008; Thorne and Frank, 2009). Although the impact of boot-defoliation on SLA was weak, we found much smaller values in May than in October 2008 for both treatments. We speculate that SLA may have increased in the fall in response to declining irradiance. The substantial temporal difference in SLA suggests that there may be considerable plasticity for this trait. As high-SLA species may be more successful in resource-rich environments (Reich et al., 1999; Wilson et al., 1999), greater plasticity for SLA would be valuable for rangelands with unpredictable climates or variable grazing regimens.

We found that boot-defoliation response in the less-sensitive species (E. wawawaiensis) was driven by tiller number and tiller mass, rather than SLA, while tiller number was the sole driver in the more-sensitive species (P. spicata). Across genotypes of all eight populations, variation for shoot biomass production following boot-defoliation was more closely related to tiller number than to any other measured variable. Thus our data better conform to the tiller demography hypothesis for defoliation tolerance than to the SLA hypothesis. The importance of tiller mass and particularly tiller number, as predictors of defoliation tolerance reported here justifies further comparative studies of these two native species for tiller demography.

Mueller and Richards (1986) have previously shown that P. spicata (= Agropyron spicatum [Pursh] Scribn. & Smith), is not constrained in terms of numbers of viable axillary buds available to elongate into new tillers, relative to its much more grazing-tolerant relative, A. desertorum (Fisch. ex Link) Schult. Hence they hypothesized that regrowth of P. spicata following clipping must be limited by a physiological limitation of bud elongation. Our results can be considered to be in agreement with Mueller and Richards' (1986) conclusion that the ability to stimulate bud elongation following defoliation, thereby generating new tillers, is critical to reestablishing the photosynthetic area that is essential to sustain growth and recover from defoliation (Richards and Caldwell, 1985). A new finding reported here, however, is that tiller mass plays an important role above and beyond that of tiller number in the recovery of E. wawawaiensis plants from boot defoliation. This means that biomass production of E. wawawaiensis is not as strongly limited by tiller number as P. spicata, both under undefoliated and defoliated conditions.

Variation in Relationships Among Traits Differed Between Species

On the basis of earlier reports (Reich et al., 1999; Wilson et al., 1999), we expected a positive correlation between SLA and boot-defoliated shoot biomass. Instead, we found no such correlation in either treatment in either species. In the absence of disturbance, species with high relative growth rate and SLA may have low absolute growth (Reich et al., 1998, 2003). A negative relationship between boot-

defoliation tolerance and control shoot biomass suggests the presence of a cost-of-tolerance, possibly related to growth investments in below-ground biomass (Rotundo and Aguiar 2008; Stowe et al., 2000; Strauss and Agrawal, 1999). However, A. desertorum, an exotic grass widely used in the Intermountain West and closely related to our two native species, invests less in below-ground biomass than P. spicata and yet is more boot-defoliation tolerant (Caldwell et al., 1981). On the basis of the cost-of-tolerance hypothesis, if control shoot biomass represents absolute growth, a negative correlation between control shoot biomass and boot-defoliation tolerance should be present. Indeed, in both species we found a strong negative correlation between these two variables (-0.564, $P < 0.000$ for P. spicata and -0.611, $P < 0.000$ for E. wawawaiensis), suggesting a cost-of-tolerance. Although below-ground investments of E. wawawaiensis remain largely unknown, its boot-defoliation tolerance may make E. wawawaiensis desirable for restoration practice under conditions of livestock grazing.

Plant basal area is consistently associated with plant biomass and vigor (Butler and Briske, 1988). In our study, plant basal area for the boot-defoliation treatment was positively correlated with boot-defoliation tolerance, suggesting that maintenance of high plant basal area is associated with greater boot-defoliation tolerance.

Intraspecific Variation for Functional Traits

Interspecific variation can be attributed mainly to evolutionary selective pressures. On the other hand, intraspecific variation in traits is a consequence of genetic, developmental, and environmental factors (Albert et al., 2011; Coleman et al., 1994) that allow species to adapt to environmental change. For example, greater variability might ensure success under changing climatic conditions. This study provided an excellent opportunity to inspect both interspecific and intraspecific variation in traits.

We found interpopulation variation within P. spicata for SLA, plant basal area, and tiller number, whereas E. wawawaiensis displayed none. For example, the tetraploid P. spicata population, P-27t, was lower than the diploid P. spicata populations for SLA, tiller number, and plant basal area. Once dominant on millions of hectacres of semiarid grasslands and sagebrush sites, P. spicata's broad distribution may be responsible for such population differentiation. On the other hand, the narrow distribution of E. wawawaiensis has perhaps limited its differentiation. Although higher trait variation will be important for rangeland species, particularly under increasingly unpredictable climatic conditions, lack of variation might limit the use of E. wawawaiensis for restoration plant material. However, in our study, the superior defoliation tolerance of E. wawawaiensis under the intense selection pressure of boot-stage defoliation might suggest otherwise, particularly since E. wawawaiensis has also been known to display superior drought-stress tolerance (Ray-Mukherjee et al., 2010). In addition, because both P. spicata and E. wawawaiensis fall within the same functional guild, increasing the mean and the variance of trait values within the guild will be of utmost significance in restoration.

Implications

Bluebunch wheatgrass (P. spicata) has long been known to be highly susceptible to livestock grazing. The well-known work of Martyn Caldwell, Jim Richards, and their colleagues linked this susceptibility to the apparent inability of this grass to initiate new tillers in response to defoliation (Caldwell et al., 1981; Richards and Caldwell, 1985; Richards et al., 1988). Later work by our group (Jones and Nielson, 1997) established that another Intermountain native species, Snake River wheatgrass (E. wawawaiensis), possesses greater defoliation tolerance than bluebunch wheatgrass. Here, boot-defoliation reduced tiller number of Snake River wheatgrass 40%, considerably less than the 58% reduction experienced by bluebunch wheatgrass. This suggests that tillering response is a primary mechanism

driving superior boot-defoliation tolerance in Snake River wheatgrass. In Snake River wheatgrass, unlike bluebunch wheatgrass, high tiller mass also seems to serve as a mechanism for production of shoot biomass following boot-defoliation. Thus the degree of Snake River wheatgrass's boot-defoliation tolerance is related to its ability to produce more and heavier tillers following defoliation. Consequently, the seeding of Snake River wheatgrass on rangelands that will be exposed to livestock grazing can be recommended on the basis of our findings.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.rama.2015.07.011.

References

Adler, P.B., Milchunas, D.G., Lauenroth, W.K., Sala, O.E., Burke, I.C., 2004. Functional traits of graminoids in semi-arid steppes: a test of grazing histories. J. Appl. Ecol. 41, 653–663.

Adler, P.B., Milchunas, D.G., Sala, O.E., Burke, I.C., Lauenroth, W.K., 2005. Plant traits and ecosystems grazing effects: comparison of U.S. sagebrush steppe and Patagonia steppe. Ecol. Appl. 15, 774–792.

Albert, C.H., Grassein, F., Schurr, F.M., Vieilledent, G., Violle, C., 2011. Why and how should intra-specific variability be considered in trait-based plant ecology? Perspect. Plant Ecol. Evol. Syst. 13, 217–225.

Belsky, A.J., 1986. Does herbivory benefit plants? A review of the evidence. Am. Nat. 127, 870–892.

Blaisdell, J.P., Pechanec, J.F., 1949. Effects of herbage removal at various dates on vigor of bluebunch wheatgrass and arrowleaf balsamroot. Ecology 30, 298–305.

Butler, J.L., Briske, D.D., 1988. Population structure and tiller demography of the bunchgrass *Schizachyrium scoparium* in response to herbivory. Oikos 51, 306–312.

Caldwell, M.M., Richards, J.M., Johnson, D.A., Nowak, R.S., Dzurec, R.S., 1981. Coping with herbivory: photosynthetic capacity and resource allocation in two semi-arid *Agropyron* bunchgrasses. Oecologia 50, 14–24.

Carlson, J.R., Barkworth, M.E., 1997. *Elymus wawawaiensis*: a species hitherto confused with *Pseudoroegneria spicata* (Triticeae, Poaceae). Phytologia 83, 312–330.

Coleman, J.S., McConnaughay, K.D.M., Ackerly, D.D., 1994. Interpreting phenotypic variation in plants. Trends Ecol. Evol. 9, 187–191.

Coley, P.D., Bryant, J.P., Chapin III, F.S., 1985. Resource availability and plant anti-herbivore defense. Science 230, 895–899.

Cook, C.W., Stoddart, L.A., 1963. The effect of intensity and season of use on the vigor of desert range plants. J. Range Manag. 16, 315–317.

Cornelissen, J.H.C., Lavorel, S., Garnier, E., Diaz, D., Buchmann, N., Gurvich, D.E., Reich, P.B., ter Steege, H., Morgan, H.D., van der Heijden, M.G.A., Pausas, J.G., Poorter, H., 2003. A handbook of protocols for standardized and easy measurement of plant functional traits worldwide. Aust. J. Bot. 51, 335–380.

Del-Val, E., Crawley, M.J., 2005. What limits herb biomass in grasslands: competition or herbivory? Oecologia 142, 202–211.

Diaz, S., Cabido, M., Marcelo, Z., Carretero, M., Julieta, E., Julieta, A., 1999. Plant functional traits, ecosystem structure and land-use history along a climatic gradient in central-western Argentina. J. Veg. Sci. 10, 651–660.

Diaz, S., Noy-Meir, I., Cabido, M., 2001. Can grazing response of herbaceous plants be predicted from simple vegetative traits? J. Appl. Ecol. 38, 497–508.

DiTomaso, J.M., 2000. Invasive weeds in rangelands: species, impacts, and management. Weed Sci. 48, 255–265.

Fleischner, T.L., 1994. Ecological costs of livestock grazing in western North America. Conserv. Biol. 8, 629–644.

Ganskopp, D., 1988. Defoliation of Thurber needlegrass: herbage and root responses. J. Range Manag. 41, 472–476.

Garnier, E., Shipley, B., Roumet, C., Laurent, G., 2001. A standardized protocol for the determination of specific leaf area and leaf dry matter content. Funct. Ecol. 15, 688–695.

Grime, J.P., 2001. Plant strategies, vegetation processes, and ecosystems properties. John Wiley & Sons Ltd., Chichester, England, UK 456 pp.

Guo, Y., Wang, D., Ba, L., Bai, Y., Liu, B., 2008. Interactions between herbivory and resource availability on grazing tolerance of *Leymus chinensis*. J. Exp. Bot. 63, 113–122.

Jones, T.A., 2003. The restoration gene pool concept: beyond the native versus non-native debate. Restor. Ecol. 281–290.

Jones, T.A., Nielson, D.C., 1997. Defoliation tolerance of bluebunch and Snake River wheatgrasses. Agron. J. 89, 270–275.

Mack, R.N., Thompson, J.N., 1982. Evolution in steppe with few large, hooved mammals. Am. Nat. 119, 757–773.

Maschinski, J., Whitham, T., 1989. The continuum of plant responses to herbivory, the influence of plant association, nutrient availability, and timing. Am. Nat. 134, 1–19.

McNaughton, S.J., 1983. Compensatory plant growth as a response to herbivory. Oikos 40, 329–336.

McNaughton, S.J., 1984. Grazing lawns: animals in herds, plant form, and coevolution. Am. Nat. 124, 863–886.

McNaughton, S.J., 1985. Ecology of a grazing ecosystem: the Serengeti. Ecol. Monogr. 55, 259–294.

Milchunas, D.G., Sala, O.E., Lauenroth, W.K., 1988. A generalized model of the effects of grazing by large herbivores on grassland community structure. Am. Nat. 132, 87–106.

Monsen, S.B., Stevens, R., Shaw, N.L., 2004. Restoring western ranges and wildlands. RMRS-GTR 136 2. U.S. Forest Service Gen. Tech. Rep, Fort Collins, CO, USA, pp. 338–342.

Morrison, K.J., Kelley, C.A., 1981. Secar bluebunch wheatgrass. Washington State University, EB 0991, Pullman, WA, USA 6 pp.

Mueggler, W.F., 1972. Influence of competition on the response of bluebunch wheatgrass to clipping. J. Range Manag. 25, 88–95.

Mueller, R.J., Richards, J.H., 1986. Morphological analysis of tillering in *Agropyron spicatum* and *Agropyron desertorum*. Ann. Bot. 58, 911–921.

Nimon, K., Oswald, F.L., Roberts, J.K., 2013. Interpreting regression effects. R package version 2.0-0. Available at:, http://cran.r-project.org/web/packages/yhat/index.html Accessed 29 September, 2015.

Ogle, D., 2002. Plant fact sheet: Snake River wheatgrass. Available at:, http://plants.usda.gov/factsheet/pdf/fs_elwa2.pdf Accessed 29 September, 2015.

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, USA, pp. 67–100.

Poorter, H., Niinemets, U., Poorter, L., Wright, I., Villar, R., 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytol. 182, 565–588.

R Development Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna Available at: http://www.R-project.org.

Ray-Mukherjee, J., Jones, T.A., Adler, P.B., Monaco, T.A., 2010. Drought tolerance in two native perennial bunchgrasses used for restoration in the Intermountain West, USA. Plant Ecol. 212, 461–470.

Ray-Mukherjee, J., Jones, T.A., Adler, P.B., Monaco, T.A., 2011. Immature seedling growth of two North American native perennial bunchgrasses and the invasive grass *Bromus tectorum*. Rangel. Ecol. Manag. 64, 358–365.

Ray-Mukherjee, J., Nimon, K., Mukherjee, S., Morris, D., Slotow, R., Hamer, M., 2014. Using commonality analysis in multiple regressions: a tool to decompose regression effects in the face of multicollinearity. Methods Ecol. Evol. 5, 320–328.

Reich, P.B., Tjoelker, M.G., Walters, M.B., Wanderklein, 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. Funct. Ecol. 12, 327–338.

Reich, P.B., Ellsworth, D.S., Walters, M.B., Vose, J.M., Gresham, C., Volin, J.C., Bowan, W.D., 1999. Generality of leaf trait relationships: a test across six biomes. Ecology 80, 1955–1969.

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 strategies. Int. J. Plant Sci. 164, S143–S164.

Richards, J.H., Caldwell, M.M., 1985. Soluble carbohydrates, concurrent photosynthesis and efficiency in regrowth following defoliation: a field study with *Agropyron* species. J. Appl. Ecol. 22, 904–920.

Richards, J.H., Mueller, R.J., Mott, J.J., 1988. Tillering in tussock grasses in relation to defoliation and apical bud removal. Ann. Bot. 62, 173–179.

Rotundo, J.L., Aguiar, M.R., 2008. Herbivory resistance traits in populations of *Poa ligularis* subjected to historically different sheep grazing pressure in Patagonia. Plant Ecol. 194, 121–133.

Ruiz, R.N., Ward, D., Saltz, D., 2008. Leaf compensatory growth as a tolerance strategy to resist herbivory in *Pancratium sickenbergeri*. Plant Ecol. 198, 19–26.

SAS Institute, 2008. SAS/STAT user's guide. Version 9.2. SAS Institute, Inc., Cary, NC, USA 707 pp.

Sheley, R.L., Olson, B.E., Larson, L.L., 1997. Effect of weed seed rate and grass defoliation level on diffuse knapweed. J. Range Manag. 50, 39–43.

Sheley, R.L., Bingham, B.S., Svejcar, T.J., 2008. Crested wheatgrass defoliation intensity and season on medusahead invasion. Rangel. Ecol. Manag. 61, 211–217.

Stowe, K.A., Marquis, R.J., Hochwender, C.G., Simms, E.L., 2000. The evolutionary ecology of tolerance to consumer damage. Annu. Rev. Ecol. Syst. 31, 565–595.

Strauss, T., Agrawal, A.A., 1999. The ecology and evolution of plant tolerance to herbivory. Trends Ecol. Evol. 14, 179–185.

Thorne, M.A., Frank, D.A., 2009. The effects of clipping and soil moisture on leaf and root morphology and root respiration in two temperate and two tropical grasses. Plant Ecol. 200, 205–215.

Tiffin, P., Rausher, M.D., 1999. Genetic constraints and selection acting on tolerance to herbivory in the common morning glory *Ipomea purpurea*. Am. Nat. 154, 700–716.

van der Heyden, F., Stock, W.D., 1996. Regrowth of a semiarid shrub following simulated browsing: the role of reserve carbon. Funct. Ecol. 10, 647–653.

van der Meijden, E., Wijn, M., Verkaar, H.J., 1988. Defence and regrowth, alternative plant strategies in the struggle against herbivores. Oikos 51, 355–363.

Violle, C., Enquist, B.J., McGill, B.J., Jiang, L., Albert, C.H., Hulshof, C., Jung, V., Messier, J., 2012. The return of the variance: intraspecific variability in community ecology. Trends Ecol. Evol. 27, 244–252.

Wilson, A.M., Harris, G.A., Gates, D.H., 1966. Cumulative effects of clipping on yield of bluebunch wheatgrass. J. Range Manag. 19, 90–91.

Wilson, P.J., Thompson, K., Hodgson, J.G., 1999. Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. New Phytol. 143, 155–162.