Indications of transgenerational phenotypic plasticity for morphological traits in *Bromus tectorum* under a two-generation reciprocal drought experiment

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Invasive plant species such as *Bromus tectorum* pose a substantial threat to low-resilience ecosystems such as the sagebrush steppe in the North American intermountain west. Determining the extent to which *B. tectorum* expresses transgenerational morphological plasticity in response to drought is necessary for understanding how rapidly the species may acclimate to novel climates and colonize new environments. We investigated transgenerational plasticity of stomatal density, root and shoot length, and root and shoot length ratio across replicates of four genotypes reciprocally subjected to two generations of high and low watering regimes. Average stomatal density of offspring of water-limited parents did not differ based on whether they received high or low water treatments. Well-watered offspring of parents from the low water treatment had significantly longer roots and shoots than water-limited offspring. Water-limited offspring of parents from the low watering treatment displayed greater root : shoot lengths than did well-watered offspring. Additionally, genetic variation for phenotypic plasticity was detected for root and shoot length as well as the ratio of root : shoot length, but not for stomatal density. We conclude that *B. tectorum* is phenotypically plastic in response to drought and furthermore displays complex tradeoffs and transgenerational phenotypic plasticity for morphological traits, which together may influence both how the species will cope with climate change and our ability to control the species.

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ABSTRACT

Invasive plant species such as *Bromus tectorum* pose a substantial threat to low-resilience ecosystems such as the sagebrush steppe in the North American intermountain west. Determining the extent to which *B. tectorum* expresses transgenerational morphological plasticity in response to drought is necessary for understanding how rapidly the species may acclimate to novel climates and colonize new environments. We investigated transgenerational plasticity of stomatal density, root and shoot length, and root and shoot length ratio across replicates of four genotypes reciprocally subjected to two generations of high and low watering regimes. Average stomatal density of offspring of water-limited parents did not differ based on whether they received high or low water treatments. Well-watered offspring of parents from the low water treatment had significantly longer roots and shoots than water-limited offspring. Water-limited offspring of parents from the low watering treatment displayed greater root : shoot lengths than did well-watered offspring. Additionally, genetic variation for phenotypic plasticity was detected for root and shoot length as well as the ratio of root : shoot length, but not for stomatal density. We conclude that *B. tectorum* is phenotypically plastic in response to drought and furthermore displays complex tradeoffs and transgenerational phenotypic plasticity for morphological traits, which together may influence both how the species will cope with climate change and our ability to control the species.

INTRODUCTION

Exotic plants have displaced native plant communities on vast tracts of land in the intermountain west of the United States, resulting in changes to soil physical, biological, and chemical properties, and alteration of ecosystem processes like water and nutrient cycling (D’Antonio & Vitousek 1992, Wilcox et al. 2012, Weber 2015). Concomitant increases of wildfire severity and frequency (Levine et al. 2003) and reduced productivity of crops and rangelands resulted in an estimated loss of $27.9 billion per annum in the early 2000’s (Pimentel et al. 2001), amounting to over $40.1 billion in losses in 2019 (assuming a 43.75% cumulative inflation rate; U.S. Bureau of Labor Statistics). Furthermore, in 2013 alone the U.S. government spent over $1.7 billion on prevention, detection, and management of invasive species (Venn et al. 2016).

Mean temperature, and the timing and quantity of precipitation are changing locally and globally because of increasing atmospheric greenhouse gas concentrations (IPCC 2007, Mote and Salathe 2010). Evidence from climate change research suggests that the northern third of North America will experience increased winter precipitation (between +8% and +42%), decreased spring and summer precipitation (mean of -13%), and a mean annual temperature increase ranging from 1.6° and 5.4° C (Mote and Salathe 2010, Bradley 2009). In the short-term, plant populations are expected to respond to climate change through a combination of shifts in species distributions and acclimation through phenotypic plasticity, i.e., the capacity of an organism to express quantitatively or qualitatively distinct phenotypes based on environmental conditions (Abatzglou and Kolden 2011, Aronson and McNulty 2009, Bradley 2009, Nicotra *et al*. 2010, Smith *et al.* 2009, Sultan 2003).

The impacts of exotic plants vary with the susceptibility of invaded systems (Richards *et al.* 2006, Bakker *et al.* 2013, Chambers et al. 2014) and the characteristics of the exotics themselves, including the potential for pronounced phenotypic plasticity (Rejmánek and Richardson 1996, Lake and Leishman 2004). Coincident with shifting species distributions, the present global shift toward a warmer and more arid climate potentiates irreversible conversion of diverse vegetation communities to grasslands that may harbor fewer plant species (McDowell N. pers. comm.). In the case of the intermountain west of the United States (hereafter "intermountain west"), invasion by the exotic annual grass *Bromus tectorum* L. (cheatgrass) reduces species richness of both plant (Bangert and Huntly 2010) and animal communities (Ostoja and Schupp 2009), thus compromising species-dependent ecosystem services (Chambers *et al.* 2014). For instance, cheatgrass invasion has been shown to replace the availability of nutritionally important native seed resources with seeds of inferior nutritional quality (Levine et al. 2003). In the sagebrush steppe of western North America, however, the most significant aspect of cheatgrass invasion is the reduction of the fire return interval from about 60 years to less than 5 years (Abatzglou and Kolden 2011, Bradley 2009, D'Antonio and Vitousek 1992, Levine et al 2003). All of these issues are likely to be exacerbated by rising global temperature regimes and altered patterns of precipitation (Bradley 2009; Breshears et al. 2013; Rice and Mack 1991$_{a,b,c}$; Stuart and Hull 1949). Thus, understanding how *B. tectorum* will behave in low-resilience ecosystems, such as sagebrush steppe in the intermountain west under varied precipitation regimes has become a major priority for land managers and remains a substantial research challenge to ecologists (Snyder et al. 2019).

B. tectorum has spread across much of the United States since its introduction in the late 1800s (EDMapS 2019). The species is notoriously abundant in the intermountain west where its rapid growth and high reproductive capacity have allowed it to dominate about 200,000 km^2 of rangeland (Novak and Mack 2001, Wade 2015). *B. tectorum* invasion causes both subtle and drastic ecosystem alterations, including: trophic structure modifications via altered structure and diminished diversity of soil microbes (Weber et al. 2015, Weber 2015, Gehring et al. 2016), replacement of nutritious native seed sources with seed of inferior nutritional quality (Levine *et al*. 2003), and reduction of the fire return interval in sagebrush steppe of the American west from about 60 years to less than 5 years (Abatzglou and Kolden 2011, Bradley 2009, D'Antonio and Vitousek 1992, Levine *et al.* 2003). Collectively, these alterations generally preclude the re-establishment of native perennial plants and generate feedback loops favoring fire-adapted cheatgrass propagules (Baynes *et al.* 2012, Bradley 2009).

Like many other obligate self-fertilizing species, *B. tectorum* exhibits high among-population genetic diversity (G_{ST} = 0.478; Novak et al. 1991), but lower within-population genetic variation than most diploid seed plants (only 4.6% of loci were found to be polymorphic per population; Novak et al. 1991). These characteristics, coupled with the ability of *B. tectorum* to rapidly spread to new and diverse environments, suggest that phenotypic plasticity in *B. tectorum* may be a particularly important mechanism for responding to environmental variability (Novak et al. 1991, Rice and Mack 1991$_a$). A meta-analysis investigating the correlation between invasiveness and single-generation phenotypic plasticity for morphological and ecophysiological plant traits concluded that phenotypic plasticity did not account for invasiveness (Palacio-López and Gianoli 2011). However, the authors suggested that their negative results were potentially due to the ephemerality of plasticity in the traits considered in their meta-analysis and the likelihood that different traits differentially confer invasiveness in different environments. Furthermore, the 35 studies included in their meta-analysis collectively addressed only 14 plant traits. Weak correlations detected between invasiveness and plasticity may thus be due to considering an insufficient number of plant traits.

Transgenerational phenotypic plasticity occurs when exposure of an ancestral generation to environmental stressors influences the environmental-dependence of phenotypic expression in progeny (Herman and Sultan 2011, Engqvist and Reinhold 2016). Considering the possibility of transgenerational phenotypic plasticity in traits of *B. tectorum* addresses important questions concerning how this species may respond to emergent selective pressures across generations, and how repeatedly adaptive plasticity in organismal developmental programs may become fixed into genomes and thereby influence the long-term persistence of populations (Lande et al. 2009). For instance, a transgenerational reciprocal transplant study of an annual forb (*Polygonum persicaria*), that imposed soil moisture treatments on both parental and grandparental generations showed that progeny traits related to survival during drought were significantly influenced by ancestral exposure to drought stress (Herman et al. 2012). Specifically, progeny of grandparent and parent plants responded to limiting levels of soil moisture by producing greater biomass, longer root systems, and maintaining higher survival than progeny without a genetic history of drought exposure (Herman et al. 2012). Importantly, the effects of grandparental and parental moisture treatments in this study were both independently and cumulatively significant, wherein progeny from drought-stressed parents *and* grandparents showed greater biomass and root length than progeny from drought stressed parents, but well-watered grandparents, or vice-versa. This result fits an *adaptive matching* scenario, in which exposure of plants from a parental (*P*) generation to stressful environmental conditions, results in F_1 plants that express a genotype-dependent phenotype that confers a selective advantage when F_1 plants are exposed to the stress imposed on the *P* generation. Interestingly, the same “matched/mismatched” experiment conducted on *Polygonum hydropiper* yielded opposite results, i.e., a *maladaptive* trend wherein exposure of *P* generation plants to stressful environmental conditions results in genotype-dependent F_1 plant phenotypes that perform more poorly in all environments (Herman and Sultan 2011).

The ability to generate a rapid, contextually appropriate phenotypic response to a stimulus based upon an earlier sensitization of a response pathway could confer unique advantages to an organism faced with variable abiotic or biotic perturbations (Hilker et al. 2016). Despite potential management implications for invasive species, the occurrence of transgenerational plasticity in exotic plants remains relatively unexplored. In a reciprocal sowing study, Rice and Mack (1991_a) explored how genetic variation was partitioned for various traits of *B. tectorum* and found that flowering time varies by source population but not by genotype in a given environment. Early anthesis of *B. tectorum* plants displayed little variation within populations or within genetic lines (families), and is surely a critical population-level attribute of cheatgrass survival in habitats with unpredictable spring and summer precipitation (Rice and Mack $1991_{a,b,c}$). Unfortunately, most studies of phenotypic plasticity have sought to detect plasticity using a limited number of plant traits, and within only a single generation. However, only by considering the plasticity of multiple integrated traits across generations can phenotypic adaptations related to plant function under water limitation be fully understood (Forsman 2015).

In this study, we use a partial factorial design to investigate whether F_1 generation *B. tectorum* plants under high-H_2O and low-H_2O regimes express divergent quantitative morphological measures based upon the watering regime their parents experienced. The traits in question include those important to nutrient and water acquisition (*i.e.* root length), transpiration and carbon assimilation (*i.e.* stomatal density), and morphological tradeoffs between belowground and aboveground biomass (assessed via shoot length and root-to-shoot length ratio). We hypothesize that (1) F_1 plants exposed to drought-like conditions will display morphological tradeoffs between above and belowground biomass that enables increased water acquisition and reduced water loss via transpiration, and (2) greater trait plasticity in response to H_2O availability will be observed in F_1 plants of parents that were exposed to drought-like conditions than in progeny of well-watered parents.

MATERIALS AND METHODS

Field Collection

Seed from 2013 seed crops was collected from wild *B. tectorum* populations in and around Pocatello, ID, USA in April and May 2014. The native plant communities of the region are representative of large tracts of rangeland in the intermountain west, *i.e.* sagebrush steppe (NVC Type G304) and juniper woodlands (NVC Type G247). Seven distinct areas supporting *B. tectorum*, separated by non-anthropogenic barriers to propagule transmission, served as the seed source populations from which seeds were collected. Selected *B. tectorum* seed-plants within source populations were at least fifteen paces apart (~15 meters) from one another. Because *B. tectorum* seeds seldom travel more than two meters from parent plants (McCarlie *et al.* 2001, Rice & Mack 1991$_{a,b,c}$), each plant from which seed was collected was assumed to harbor seed of a distinct genotype within each population.

Greenhouse and Data Collection:

Forty individuals of each wild parent plant were grown from collected seed, constituting the parental generation (*P*). Seed from *P* generation plants were germinated and grown under identical conditions on two benches in a glasshouse to generate a generation of full-sibling filial (F_1) stock seed with a reduced influence of seed-source location (Dyer et al. 2010). All plants were grown in steam-sterilized soil sourced from one location in the sagebrush steppe near Pocatello, ID, in 2.83 liter 'TP414' plastic propagation pots (Stuewe Sons Inc., Corvallis, OR). Greenhouse temperatures were allowed to range from 19° C during the night to 29° C during the afternoon. A combination of artificial (PPFD = 375 μmol m^{-2} s^{-1}) and ambient lighting was distributed as evenly as possible across all plants, which were systematically rotated positionally twice weekly on the glasshouse benches to avoid glasshouse microclimate effects. Application of 10 mL volumes of water via top-watering, and daily monitoring of throughout the experiment ensured consistency of the following two watering treatments: (1) 30% - 40% volumetric water content (hereafter "high-H_2O"), and (2) 14% - 18% VWC (hereafter "low-H_2O"; 14% VWC was empirically determined to roughly correlate to the standard permanent wilting point value of -1.5 MPa for our soils (Huber D. pers. comm.). Soil moisture was monitored using EC-5 sensors (Decagon Devices, now *METER*; Pullman, WA) installed in four plant pots for each watering treatment and a *METER* ProCheck data viewer. *P* and F_1 plants were fertilized once during week number 15 of each 22-week growing cycle using a 21:5:20 (N:P:K) mix to achieve an application rate of approximately 6.5 × 10^{-4} grams N per plant. Three weeks before the end of each growing cycle, the photoperiod was extended from 12d:12n to 16d:8n, by supplying an additional four hours of artificial lighting, to force early flowering.

Low-H_2O and high-H_2O treatments were randomly applied to both: (1) individual plants from the *P* generation (up to the time that seed was harvested), and (2) F_1 offspring obtained from the *P* generation seeds. Offspring from each plant were presumed to be full-sibs due to the cleistogamous, obligate selfing mating system of *B. tectorum*. Thus, we use the term *genotype* rather than *genetic family* to refer to individuals from one parent plant, though it must be noted that full-sibs are not expected to be genetically uniform due to segregation and crossing over during meiosis.

Morphological characteristics were recorded for three random leaves for each of 102 individuals (52 plants whose parents received low-H_2O and 50 plants whose parents received high-H_2O). The leaf pseudoreplicates were averaged to obtain a single observation for each individual genotype. For each of three random leaves per plant, stomatal counts were the average stomatal number at three random locations along a 2.2 cm-long row of stomata along the center mid-portion of leaves after soaking the oldest (i.e. lowest) leaf on each plant in 0.025 M PO_4^{-3} for 48 hrs. Plants were dried at 60° C for 48 hours before measuring root and shoot length using ImageJ 1.x (Schneider et al. 2012).

In total, multiple plants representing 17 genotypes were obtained for the low-H_2O group and 19 genotypes for the high-H_2O group. Due to die-off events, however, empty cells occurred for some filial water/genotype combinations. These were eliminated from analyses. Further, treatment combinations with a single observation were eliminated due to their inordinately large effect on linear models. These steps left 37 observations from four genotypes originating from parents exposed to low-H_2O treatments and 35 observations from four genotypes originating from parents exposed to high-H_2O treatments.

Statistical Analysis:

Linear mixed-effect models were run separately for filial groups from both low-H_2O and high-H_2O parents, with filial water level (L/H) as a fixed effect and genotype as a random effect. Satterthwaite estimation for degrees of freedom for *F* statistics was used when considering fixed effects using *F*-tests, whereas likelihood ratio tests --following implementation of REML for estimation of variance components-- was used when assessing the H_0, $\sigma^2_{Genotype} = 0$, (Patterson & Thompson 1971). The statistical environment R (R Core Team, 2018) was used for all analyses, with heavy reliance on the R package lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2017).

RESULTS

Stomatal Density:

Average stomatal density of offspring from the low-H_2O parental group did not differ between high-H_2O and low-H_2O treatment levels ($F_{1,31} = 1.105$, $p = 0.301$), genotype ($X^2_1 = -0.0$, $p = 1.0$), or interaction between genotype and treatment ($X^2_1 = 0.059$, $p = 0.808$; Figure 1). For high-H_2O parents, offspring in the high-H_2O group had, on average, much higher stomatal densities than the low-H_2O group ($F_{1,29.9} = 7.23$, $p = 0.003$). Genotype ($X^2_1 = 0$, $p = 1.0$), and genotype × H_2O level interactions, however, were not significant ($X^2_1 = 0$, $p = 1.0$; Figure 1a).

Shoot Length:

For low-H_2O parents, offspring from the high-H_2O group had significantly longer shoot length than low-H_2O offspring ($F_{1,3.3} = 16.2$, $p = 0.023$; Figure 1b). Genotype ($X^2_1 = 0.177$, $p = 0.679$) and genotype × H_2O level interactions were statistically similar ($X^2_1 = 2.08$, $p = 0.149$). For the high-H_2O parents, a weak ($\alpha = 0.1$) trend was evident for high-H_2O offspring having longer shoots than low-H_2O offspring ($F_{1,2.4} = 7.17$, $p = 0.101$). Genotype ($X^2_1 = 0.288$, $p = 0.591$), and genotype × H_2O level interactions ($X^2_1 = 0.558$, $p = 0.455$; Fig. 1b) were not significant.

Root Length:

For low-H_2O parents, root length for the high-H_2O treatment were longer than root length for the low-H_2O treatment ($F_{1,30} = 4.35$, $p = 0.046$; Figure 1c). Neither genotype ($X^2_1 = 0$, $p = 1.0$) or genotype × H_2O level interactions ($X^2_1 = 1.87$, $p = 0.172$) were significantly different. For the high-H_2O parents, roots lengths were not significantly different between offspring of high-H_2O parents ($F_{1,35} = 1.42$, $p = 0.241$), genotypes ($X^2_1 = 0$, $p = 1.0$), or genotype × H_2O level interactions ($X^2_1 = 0$, $p = 1.0$; Fig. 1c).

Root-Shoot Ratio:

For low-H_2O parents, the root-shoot ratio of offspring of the low-H_2O group was greater than that of the offspring from the high-H_2O group ($F_{1,30} = 4.52$, $p = 0.042$, Fig. 1d). No differences were apparent for genotype ($X^2_1 = 0$, $p = 1.0$) or genotype × H_2O level interactions ($X^2_1 = 0.409$, $p = 0.522$; Figure 4). For high-H_2O parents, offspring treatment differences were not apparent ($F_{1,2.6} = 1.35$, $p = 0.339$), nor were differences between genotype ($X^2_1 = 0.33$, $p = 0.565$), or genotype × H_2O level interactions ($X^2_1 = 0.01$, $p = 0.930$; Fig.1d).

DISCUSSION

Longevity of Drought-stress effects:

We found that exposing a parental generation of *B. tectorum* to ample versus limiting levels of soil water availability significantly influenced the response of directionality and plasticity of F_1 generation traits when exposed to the same high-H_2O versus low-H_2O treatments. Extensive mortality in this experiment precluded a full factorial design of this experiment, which would have allowed replication of each genotype across both high-H_2O and low-H_2O during the P generation as well as across both high-H_2O and low-H_2O treatments during the F_1 generation. Instead, with the exception of one genotype (H1.8) available for all treatments (see Fig. 1), our results are based on two separate sets of genotypes: (1) a set that was exposed to high-H_2O during the P generation and both high-H_2O and low-H_2O treatments during the F_1 generation, and (2) a set that was exposed to low-H_2O during the P generation and both high-H_2O and low-H_2O treatments during the F_1 generation. However, the complete lack of statistical interaction detected between genotype and watering treatment in this study indicates that genotypes generally performed consistently to treatments within parental watering groups, and the significant effect of parental watering level upon F_1 generation phenotypes suggests that the significant influence of parental watering may be due to transgenerational plasticity acting in this system. Within this framework we found that offspring exposed to low-H_2O versus high-H_2O

regimes from parents that received low-H_2O displayed notable and significant differences between F_1 watering treatment in root length, shoot length, and root-to-shoot ratio. Conversely, stomatal density varied significantly among offspring of high-H_2O parents, but not low-H_2O parents (Figures 1a-d).

F_1 genetic variation in plasticity of stomatal density was markedly different for low-H_2O P and high-H_2O P watering regimes (Fig. 1a, Fig 2). All genotypes from high-H_2O P watering regimes displayed stomatal density reaction norms that increased when moving from low-H_2O F_1 to high-H_2O F_1 watering regimes, suggesting a potential advantage for progeny from parents without limiting water to develop greater stomatal density if they also obtain adequate water (Fig. 1a). Meanwhile, genotypes from the low-H_2O P watering regime display a wide variety of reaction norms responses when moving from low-H_2O F_1 to high-H_2O F_1 watering regimes, indicative of a "bet-hedging" strategy wherein progeny express highly variable stomatal traits (Fig. 1a). In general, mean stomatal density of low-H_2O F_1 plants from low-H_2O P plants was greater than that of low-H_2O F_1 plants from high-H_2O P plants, suggesting a potential advantage for progeny from drought-stricken parents to develop greater stomatal density if they also experience drought. The bet-hedging trend we observed via reaction norms suggests that there is no optimal allocational growth strategy for stomatal density with respect to progeny from parents that suffered drought when the F_1 generation did not. This may relate to the energetic cost associated with an increased number of guard cells (Vialet-Chabrand et al. 2017), entailing a metabolic cost that is high enough to render increased stomatal density disadvantageous until some developmental tradeoff threshold is reached (Gutschick 1999). Complex cost-benefit developmental tradeoffs associated with stomatal density may also account for the observed stochasticity of phenotypic plasticity for this trait in *B. tectorum*. For instance, a ruderal annual plant, with greater stomatal density would be better equipped to increase carbon assimilation rapidly in response to ephemeral water availability, conferring a competitive productivity advantage. On the other hand, high stomatal density may increase the likelihood that a plant will transpire too quickly and succumb to drought (Vialet-Chabrand et al. 2017). In circumstances where water is consistently available, as in the scenario where high-H_2O F_1 plants were from high-H_2O P parents, we found a strong directional reaction norm trend toward greater stomatal density. Such a strategy may underlie the ability of some *B. tectorum* populations to generate multiple generations per year when conditions allow, though interestingly it has been found previously that *B. tectorum* populations from mesic but not xeric environments display opportunistic phenological strategies for capturing ephemeral moisture and thereby enhancing fitness (Dyer et al. 2012).

Genetic variation for plasticity of shoot length was apparent for both low-H_2O P and high-H_2O P watering regimes, as evidenced by intersecting reaction norms (Fig. 1b).), although for all genotypes, reaction norm curves increased when moving from low-H_2O F_1 to high-H_2O F_1 watering regimes. The latter phenomenon likely reflects a simple growth increase in response to greater availability of water. Notably, however, we found that progeny of low-H_2O P plants exposed to ample water more readily (Fig 1b) and consistently (Fig. 2) allocate growth resources to shoot growth than do F_1 plants from high-H_2O P parents. We interpret this trend as evidence of the parental environment mediating the extent of phenotypic plasticity in offspring for shoot length. In particular, drought conditions during the parental generation appears to facilitate enhanced growth of photosynthetic tissue of F_1 plants with sufficient resources, which we expect would correlate positively with fitness of the F_1 generation.

Genetic variation for plasticity of root length was apparent for the high-H_2O P watering regime but marginal for the low-H_2O P watering regime (Fig. 1c). Only one genotype in the high-H_2O P watering regime displayed a reaction norm for root length that descended moving from low-H_2O F_1 to high-H_2O F_1 regimes. In contrast, all genotypes in the low-H_2O P regime displaying reaction norms of increasing root length when moving from low-H_2O F_1 to high-H_2O F_1 regimes, reflecting greater resource availability (Fig. 1c). We interpret this as evidence of anticipatory transgenerational plasticity that could confer a fitness advantage in the event that repeated generations were exposed to drought conditions.

Genetic variation for plasticity, as measured by coefficient of variation, was higher for root-to-shoot ratio responses than for any other measured trait (see “All F1” bars in Fig. 2). Two genotypes in the high-H_2O P regime displayed increasing root-to-shoot ratios when moving from low-H_2O F_1 to high-H_2O F_1 regimes. In contrast, progeny of low-H_2O P regime plants demonstrated decreased ratios for all genotypes (Fig. 1d). This suggests that for some genotypes the high-H_2O P regime favors proportionally greater growth allocation to root length than shoot length, while this trend may be reversed for other genotypes. This variation may occur because the benefit of high-H_2O P watering for allocating growth resources to light-harvesting shoot tissue reaches a saturation point at which plants’ abilities to gather soil-water and minerals becomes limiting to growth (Engqvist and Reinhold 2016).

Persistence for a large number of generations in similar edaphic and climatic conditions did not stabilize genotype responses to watering treatment within surviving genetic families. Rather, F_1 plants demonstrated plastic responses to water availability through shifts in biomass allocation, and further these shifts appear to demonstrate transgenerational plasticity resulting from parental watering level. Low-H_2O availability in the P generation appears to have a preparatory, and perhaps thereby adaptive, influence on F_1 generation plants by serving as a signal to prioritize proportionally greater development of belowground biomass than aboveground biomass.

Bromus tectorum in a Changing Climate:

Climate models for the western US project a transition of winter precipitation from snow to rain (Concilio et al. 2012, Prevéy and Seastedt 2015), and increased springtime precipitation coupled with increasingly arid summers (Mote and Salathe 2010) will favor proliferation and dominance of *B. tectorum* over native vegetation and increased wildfire frequency and severity (Link et al. 2006). Thus, continuing trends of increased offspring root length and root-to-shoot ratio to experimental drought, suggested here, may foretell the means by which *B. tectorum* may continue to proliferate regionally.

Our study did not seek to address the plasticity of phenology in *B. tectorum*. However, the timing of development and reproduction of plants is a major determinant of how well a species can exploit ephemeral soil moisture and temperatures that are conducive to growth and anthesis (Rice and Mack 1991a,b,c, Levine et al. 2003, McCarlie et al. 2001). Given that climate change is predicted to increase the temporal variability and frequency of precipitation events in the western USA, an improved understanding is needed regarding how *B. tectorum* phenology will respond to altered precipitation regimes. In a reciprocal sowing study, Rice and Mack (1991a) explored how genetic variation was partitioned for certain traits of *B. tectorum* and found that flowering time varies by source population but not by genotype in a given environment. Early anthesis of *B. tectorum* plants displayed little variation within populations or within genetic lines (genotypes), and is likely a critical population-level attribute of cheatgrass survival in habitats characterized by unpredictable spring and summer precipitation (Rice and Mack 1991a,b,c). Early phenology has probably been selected for to such an extent in cheatgrass that it has been fixed into the mean population genotype as an environmentally invariant phenotypic trait, thus posing interesting questions for how cheatgrass might respond to increased variability of the timing expected to occur under most climate change scenarios (Bradley 2009, Rice and Mack 1991a,b,c).

Management Considerations:

Applied studies of *B. tectorum* have not yet considered how morphological tradeoffs in response to environmental variation may influence the efficacy of the common chemical or biological control approaches studied in *B. tectorum*. While herbicide application may serve as an effective control for small populations of *B. tectorum*, the enormous extent of *B. tectorum* invasion across US rangelands and potential negative impacts to native species from off-target chemical application makes chemical control a high-cost and high-risk management option (Kyser *et al*. 2017, Lawrence 2015). The non-native perennial grass *Agropyron cristatum* used for rangeland restoration has been used as a biocontrol due to its ability to outcompete *B. tectorum* for soil nitrogen and phosphorous (Blank *et al.* 2015). Microbial biological control of *B. tectorum* using *Pyrenophora semeniperda* (Meyer *et al.* 2010) and *Pseudomonas fluorescens* (Kennedy 2017), coupled with burning and herbicide application, together were shown to achieve nearly complete eradication of *B. tectorum* in test plots (Meyer *et al.* 2010, Kennedy 2017). However, our results suggest that diverse control approaches may hold the greatest promise for controlling *B. tectorum* because of: (1) the capacity of *B. tectorum* to plastically adjust its growth in response to stress, and (2) genetic variation for trait plasticity in *B. tectorum* – which amounts to a "moving target" for control measures. However, only economically feasible control measures will ultimately be employed to manage *B. tectorum*.

We suggest that future research regarding the control of *B. tectorum* should focus on control measures that target plant organs which are likely to be easiest to target under a given environmental condition, principally drought. For instance, in this study, plants that received low-H_2O as opposed to high-H_2O during the P generation yielded greater differences between the two watering regimes applied to the F_1 generation plants for both shoot lengths and root lengths. However, we found that shoot length remained proportionally greater than root growth for plants exposed to low-H_2O in the P generation, indicating that, with increasing aridity, increased surface area of shoot tissue may become an easier target for control measures than the extent of plant surface area composed of root tissue. Managers of course must remain attentive to the need for control measures that do not negatively impact native vegetation but rather enable desired native species to re-establish themselves where their replacement of *B. tectorum* is possible. Another important direction for future research that emerges from this work concerns the way in which *B. tectorum* fecundity may be influenced by increasing aridity. If we expect climate conditions that impose multiple successive generations of water limitation to produce plants with greater photosynthetic surface area, can we expect that increase in photosynthetic capacity to increase plant fecundity? Developing a better understanding of morphological tradeoffs in *B. tectorum* in response to ecologically-relevant stressors should promote new approaches or combinations of approaches for striking a balance between the eradication of *B. tectorum* without causing undue harm to non-target biota and more accurately predicting how and where climate change might influence the fitness of *B. tectorum*.

CONCLUSIONS

Our results are indicative, but not conclusive, of a trend of potentially adaptive transgenerational plasticity of morphometric traits in *B. tectorum* in response to multi-generational imposed drought. We suggest that future studies building upon results presented here should investigate responses of a greater number of phenotypic traits in a fully-factorial experimental design to: (1) enable better prediction of long-term *B. tectorum* distributions, and (2) enable development of economically viable measure for the management of *B. tectorum* as the intermountain west becomes progressively warmer and drier. While complex and difficult to assess, investigating transgenerational phenotypic plasticity in response to simultaneous interactions of multiple environmental changes would enhance the ecological relevance of controlled studies, and provide important insight into how species might respond to climate change.

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Figure Legends:

Figure 1. *B. tectorum* phenotypic offspring characteristics with respect to parental and offspring watering treatments (a) stomatal density (b) shoot length (c) root length, and (d) root:shoot ratio. Bar graphs show measurement averages for offspring grown in low-H_2O and high-H_2O treatments. Line graphs in gray boxes show measurement averages of offspring by genetic family across water treatments for low water parents (red border) and highwater parents (blue border). Ciphers in bar graphs correspond to the null hypothesis that the true mean offspring difference (High - Low) for a response = 0. ** = significant at alpha = 0.01, * = significant at alpha = 0.05, "." = significant at alpha = 0.1. Errors bars represent standard errors.

Figure 2. Coefficient of variation measures for measured traits. Red and blue text coloring denotes low and high water treatments, respectively. Horizontal lines in text indicate parental conditioning. For instance, $F_1 - F_1|P$ indicates F_1 high water minus low water trait difference for progeny of high water parents.

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Figure 1

B. tectorum phenotypic offspring characteristics with respect to parental and offspring watering treatments (a) stomatal density (b) shoot length (c) root length, and (d) root:shoot ratio.

Figure 1. *B. tectorum* phenotypic offspring characteristics with respect to parental and offspring watering treatments (a) stomatal density (b) shoot length (c) root length, and (d) root:shoot ratio. Bar graphs show measurement averages for offspring grown in low-H_2O and high-H_2O treatments. Line graphs in gray boxes show measurement averages of offspring by genetic family across water treatments for low water parents (red border) and highwater parents (blue border). Ciphers in bar graphs correspond to the null hypothesis that the true mean offspring difference (High - Low) for a response = 0. ** = significant at alpha = 0.01, * = significant at alpha = 0.05, "." = significant at alpha = 0.1. Errors bars represent standard errors.

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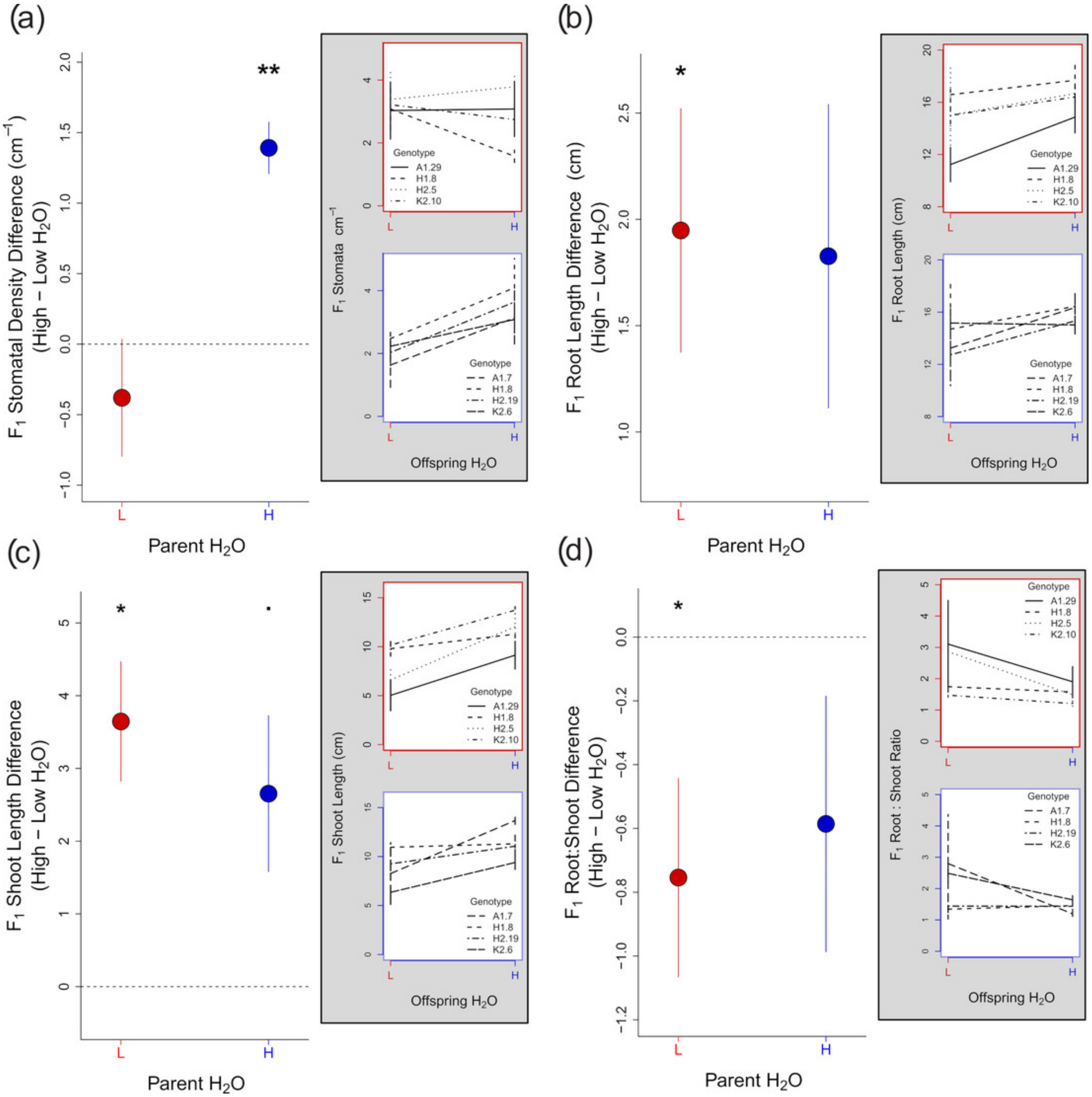
(a)
**
F_1 Stomatal Density Difference (cm^{-1}) (High − Low H_2O)
2.0
1.5
1.0
0.5
0.0
−0.5
−1.0
L
H
Parent H_2O
F_1 Stomata cm^{-1}
Genotype
A1.29
H1.8
H2.5
K2.10
Genotype
A1.7
H1.8
H2.19
K2.6
Offspring H_2O
(b)
*
F_1 Root Length Difference (cm) (High − Low H_2O)
2.5
2.0
1.5
1.0
L
H
Parent H_2O
F_1 Root Length (cm)
Genotype
A1.29
H1.8
H2.5
K2.10
Genotype
A1.7
H1.8
H2.19
K2.6
Offspring H_2O
(c)
*
.
F_1 Shoot Length Difference (High − Low H_2O)
5
4
3
2
1
0
L
H
Parent H_2O
F_1 Shoot Length (cm)
Genotype
A1.29
H1.8
H2.5
K2.10
Genotype
A1.7
H1.8
H2.19
K2.6
Offspring H_2O
(d)
*
F_1 Root:Shoot Difference (High − Low H_2O)
0.0
−0.2
−0.4
−0.6
−0.8
−1.0
−1.2
L
H
Parent H_2O
F_1 Root : Shoot Ratio
Genotype
A1.29
H1.8
H2.5
K2.10
Genotype
A1.7
H1.8
H2.19
K2.6
Offspring H_2O

Figure 2

Coefficient of variation measures for measured traits.

Figure 2. Coefficient of variation measures for measured traits. Red and blue text coloring denotes low and high water treatments, respectively. Horizontal lines in text indicate parental conditioning. For instance, F_1 – F_1|P indicates F_1 high water minus low water trait difference for progeny of high water parents.

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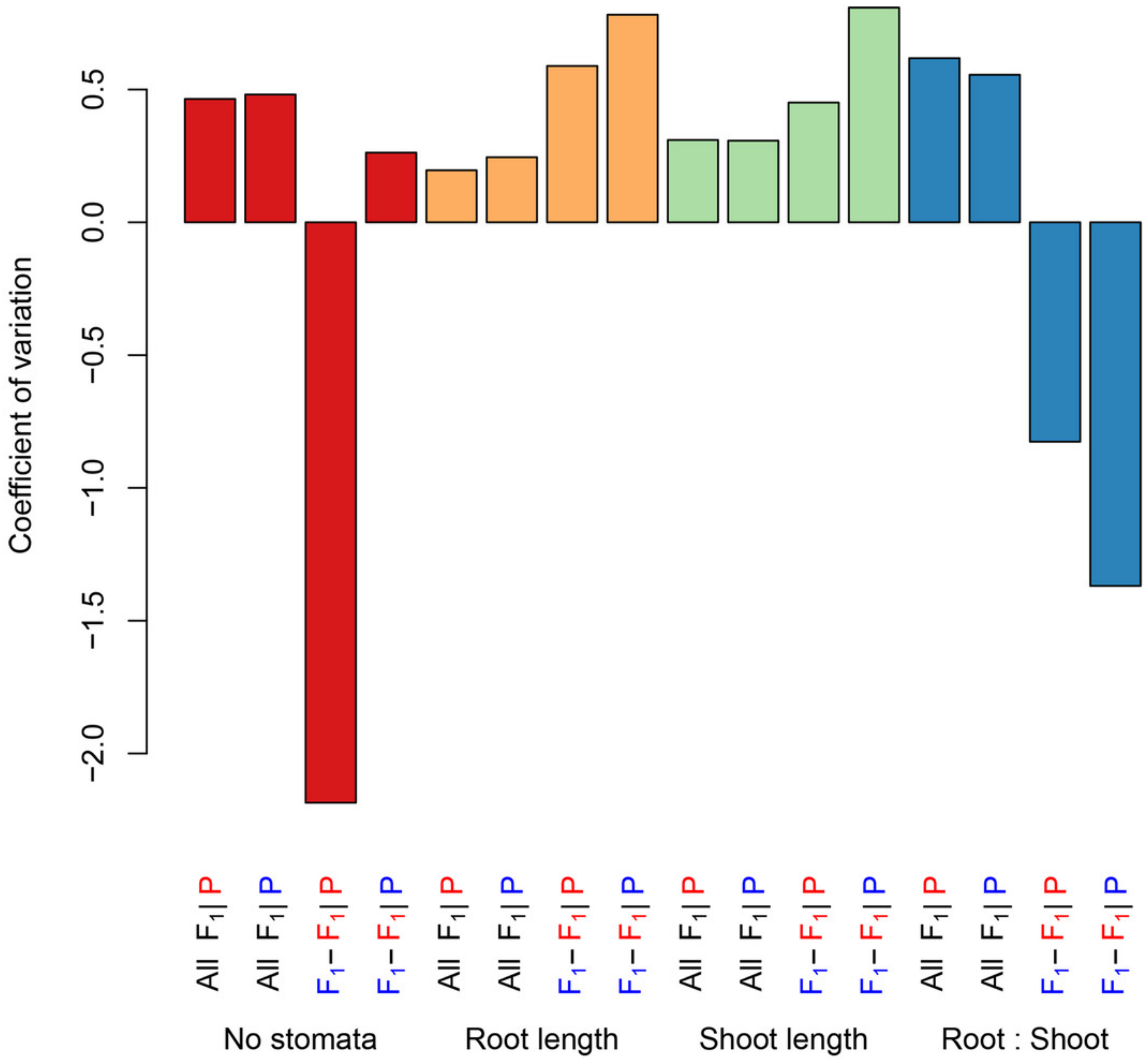
Coefficient of variation
0.5
0.0
−0.5
−1.0
−1.5
−2.0
All F1 | P
All F1 | P
F1 − F1 | P
F1 − F1 | P
No stomata
Root length
Shoot length
Root : Shoot