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Variation in phenotypic plasticity for native and invasive populations of *Bromus tectorum*

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Abstract Phenotypic plasticity is often considered important for invasive plant success, yet relatively few studies have assessed plasticity in both native and invasive populations of the same species. We examined the plastic response to temperature for *Bromus tectorum* populations collected from similar shrub-steppe environments in the Republics of Armenia and Georgia, where it is native, and along an invasive front in California and Nevada. Plants were grown in growth chambers in either ‘warm’ (30/20 °C, day/night) or ‘cold’ (10/5 °C) conditions. Invasive populations exhibited greater adaptive plasticity than natives for freezing tolerance (as measured by chlorophyll *a* fluorescence),

such that invasive populations grown in the cold treatment exhibited the highest tolerance. Invasive populations also exhibited more rapid seedling emergence in response to warm temperatures compared to native populations. The climatic conditions of population source locations were related to emergence timing for invasive populations and to freezing tolerance across all populations combined. Plasticity in growth-related traits such as biomass, allocation, leaf length, and photosynthesis did not differ between native and invasive populations. Rather, some growth-related traits were very plastic across all populations, which may help to dampen differences in biomass in contrasting environments. Thus, invasive populations were found to be particularly plastic for some important traits such as seedling emergence and freezing tolerance, but plasticity at the species level may also be an important factor in the invasive ability of *B. tectorum*.

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Photosynthesis · Plasticity index

Introduction

Phenotypic plasticity has long been considered a potentially important factor in invasive plant success (Baker 1974), and many studies have demonstrated plasticity for important traits in invasive plant populations (Davidson et al. 2011). Flexible phenotypes

may be particularly important in promoting the establishment of populations and allowing time for local adaptation (Ghalambor et al. 2007), whether at the start of a new invasion or at the “invasive front” of an existing one. However, multiple questions arise regarding plasticity within the context of biological invasions (Richards et al. 2006): (1) are invaders generally plastic as a species? or (2) is variation in plasticity within a species particularly relevant to invasive success? Until recently, most research in this area has focused on the first question using multi-species comparisons within the invasive range, whereas comparatively few studies have examined the second question by examining native and invasive populations of the same species (Bossdorf et al. 2005; Richards et al. 2006).

Plasticity in plants can be expressed for many traits and in response to many environmental variables. Yet plasticity in and of itself is not necessarily beneficial unless the response is likely to be advantageous in a particular environment. This is referred to as adaptive plasticity (Sultan 2003) and reinforces the importance of ecological and environmental context. Adaptive plasticity may contribute to invasion success by increasing survival and reproduction in certain environments and/or by maintaining relatively high levels of survival and reproduction across a range of environments (Richards et al. 2006). As the ability to be plastic is a trait itself, it is important to consider its variation within a species and to place it within the context of the immediate environment: are invasive populations relying on plasticity to succeed in a new environment, and how does this compare to populations from the native range that experience similar conditions?

By looking across both native and invasive ranges, some studies have demonstrated greater plasticity in invasive versus native populations for invaders such as *Lythrum salicaria* (Chun et al. 2007; Chun 2011), *Phalaris arundinacea* (Lavergne and Molofsky 2007), and *Senecio pterophorus* (Cano et al. 2008). However, other comparisons have not found strong differences in the plastic responses of native and invasive populations (Maron et al. 2007; Bossdorf et al. 2005; Andonian and Hierro 2011). Meimberg et al. (2010) found plasticity in flowering time for invasive *Aegilops triuncialis* that may be adaptive, but high plasticity in invasive seed production was apparently maladaptive, driven by a reduction in seed set compared to native populations. It may be difficult

to find broadly generalizable patterns on the role of plasticity in biological invasions as conclusions will necessarily depend on which environmental variables and traits are considered, and how plasticity intersects with other aspects of particular invasions. Nonetheless, a recent meta-analysis on plasticity in co-occurring invasive and non-invasive plant species (Davidson et al. 2011) found that invasive species demonstrated greater plasticity on average than non-invaders for a variety of traits. Continued research on the question of variation in plasticity for native and invasive populations of the same species is important, not only to help understand specific invasions, but to fill in the current gap in our broader understanding of the role of phenotypic plasticity in plant invasions.

Previous work on the highly invasive annual grass *Bromus tectorum* L. (“cheatgrass”) has revealed evidence for plasticity in some traits and genetic differentiation among populations for others in its invasive range in North America (Rice et al. 1992; Rice and Mack 1991). Generally, life history traits exhibited population differentiation, whereas physiological traits were more plastic. Several other studies have documented strong plastic responses to nitrogen availability for invasive *B. tectorum* compared to other species (Leffler et al. 2011; Lowe et al. 2003). This response was also seen in native populations in Hungary (Fenesi et al. 2011), but there has yet to be a coordinated study of plasticity in *B. tectorum* that simultaneously compares plants from both native and invasive ranges under the same conditions.

This study compares plasticity in native (Armenia and Georgia) and invasive (California and Nevada) populations of *B. tectorum* that grow in similar shrub-steppe ecosystems nearly halfway around the world from one another. Overall, the invasion history of *B. tectorum* has been relatively well-studied and involves multiple introductions to North America followed by considerable mixing (Novak and Mack 1993, 2001). Invasive populations are mostly likely to have come from sources in Western and Central Europe, and have invaded huge areas in the Intermountain West (Knapp 1996; Novak and Mack 2001). Of recent concern is the ability of *B. tectorum* to invade into higher elevation sites such as those at the edge of the western Great Basin and eastern Sierra Nevada (Concilio et al. 2013; Leger et al. 2009; Griffith and Loik 2010). Given this, what is the potential role of phenotypic plasticity in the ability of *B. tectorum* to invade semi-arid shrub-steppe

Table 1 *Bromus tectorum* source population information

| Region | Population | Latitude | Longitude | Elevation (m) | Mean annual temperature (°C) | Mean annual precipitation (mm) | Mean precip. in warmest quarter (mm) | Climate index | Genetic lines |
|----------|------------|----------|-----------|---------------|------------------------------|--------------------------------|--------------------------------------|---------------|---------------|
| Native | Tbilisi | 41.676 | 44.761 | 788 | 10.7 | 620 | 187 | -0.81 | 5 |
| | Surenavan | 39.800 | 44.771 | 821 | 12.9 | 262 | 52 | 1.35 | 6 |
| | Araksavan | 40.011 | 44.470 | 839 | 12.7 | 279 | 47 | 1.24 | 6 |
| | Echmiadzin | 40.169 | 44.272 | 892 | 12.1 | 299 | 62 | 1.03 | 5 |
| | Yerevan | 40.193 | 44.504 | 1,088 | 11.2 | 327 | 78 | 0.73 | 6 |
| | Avan | 40.215 | 44.557 | 1,236 | 9.8 | 359 | 85 | 0.32 | 6 |
| | Jermuk | 39.844 | 45.674 | 2,086 | 5.4 | 507 | 104 | -1.20 | 2 |
| | Meghri | 39.123 | 46.172 | 2,437 | 4.4 | 479 | 90 | -1.26 | 4 |
| Invasive | Carson | 39.197 | -119.779 | 1,447 | 9.8 | 270 | 33 | 0.75 | 6 |
| | Topaz | 38.705 | -119.553 | 1,648 | 8.4 | 357 | 42 | 0.08 | 6 |
| | Benton | 37.824 | -118.477 | 1,655 | 10.0 | 198 | 37 | 1.15 | 2 |
| | Bodie | 38.176 | -119.188 | 2,096 | 5.8 | 357 | 44 | -0.40 | 4 |
| | SNARL | 37.611 | -118.838 | 2,177 | 6.1 | 407 | 37 | -0.59 | 6 |
| | Conway | 38.074 | -119.177 | 2,367 | 4.4 | 462 | 49 | -1.16 | 5 |
| | Mammoth | 37.635 | -118.991 | 2,475 | 4.7 | 487 | 44 | -1.23 | 2 |

Temperature and precipitation data at each location were obtained from WorldClim. The climate index is the first principal component score of temperature and precipitation; low values correspond with cool and wet conditions, whereas high values correspond with warm and dry conditions

ecosystems along this invasive front? Is plasticity also prevalent and/or important for native populations that experience similar environmental conditions in Armenia and Georgia, but are otherwise unremarkable members of the plant community? Specifically, we investigated the degree of phenotypic plasticity in response to temperature for native and invasive populations of *B. tectorum* collected along elevational gradients. We examined traits related to morphology, phenology, and physiology, with the overall guiding hypothesis that adaptive plasticity in invasive populations has the potential to contribute to invasive success.

Materials and methods

Source populations

Seeds of *B. tectorum* were collected during the summer of 2006 from a total of 15 populations in Republics of Armenia and Georgia where it is native, and in California and Nevada, USA where it is invasive (Table 1, see Fig. S1 in Electronic Supplementary Material). Henceforth, we refer to the populations as

either 'native' or 'invasive'. At each population, seeds were collected from six individual plants. As *B. tectorum* is nearly exclusively autogamous (Upadhyaya et al. 1986), seeds from each plant should represent distinct genetic lines. Seeds from several lines were later found to be either infected by *Ustilago* smut or to have insufficiently-filled caryopses. Thus, each population was represented by a maximum of six lines (see Table 1). This level of replication was determined based in part on the logistical constraints of a phenotypic study involving live plants (see total experimental sample size below). Genetic analysis using microsatellite markers (part of a broader study of these populations) indicated that the native and invasive populations are genetically differentiated ($F_{st} = 0.116$). The two regions differ by an average of four private (unique) alleles per locus suggesting that there is considerable genetic separation between the native and invasive groups (Griffith, Fant, Pao, and Andonian, unpublished data).

The seeds used in the experiment presented here were collected from greenhouse-grown plants in order to minimize any maternal effects associated with population source environments. Thus, the seeds were one generation removed from the original field-

collected seeds. In February 2007, field-collected seeds were germinated and grown in a common garden at the University of California, Santa Cruz Plant Growth Facility. In order to minimize possible effects of source microbial communities, we surface-sterilized all seeds by immersing them in 10 % bleach and then 70 % ethanol for 30 s each, followed by a thorough rinse with distilled water (a preliminary trial revealed no effect of surface-sterilization on germination success; data not shown). We planted up to 15 seeds per genetic line, resulting in a total of 1,105 mature plants from which we then collected seed for the plasticity experiment. These seeds were collected during the summer and fall of 2007 and stored in paper envelopes. Prior to their use, all seeds were placed in a drying oven set to 30 °C for 2 weeks to induce after-ripening.

Population source locations

We obtained climatic data for each source population's location using the WorldClim global climate dataset (<http://www.worldclim.org>). Mean annual precipitation and temperature across population source locations were significantly correlated with each other ($r = 0.56$, $P = 0.029$). Thus, we generated a climate index (CI) using the first principal component scores of mean annual precipitation and temperature (variance explained = 78.1 %). Higher CI values correspond with hotter and drier conditions (Table 1).

We also compared the climates of the population locations used in this study to the broader native and invasive ranges of *B. tectorum* and to the Eurasian population locations examined by Novak and Mack (1993). A principal components analysis on global climate data indicated that the climates of the invasive populations in this study are more similar to the Armenian and Georgian locations used here than to the Western and Central European locations that are likely the sources of invasion (see Figs. S2 and S3 in Electronic Supplementary Material). Most of the invasive populations are located in the "Great Basin shrub steppe" ecoregion and the native populations are part of the "Eastern Anatolian montane steppe" and "Azerbaijan shrub desert and steppe" ecoregions (Olson et al. 2001). The highest elevation populations used here (Mammoth, Jermuk, and Meghri) are on the edge of temperate forest ecoregions.

Experimental design

Plants were grown in a total of four growth chambers (Conviron E15; Conviron, Inc., Winnipeg, MB, Canada) in either warm or cold conditions. Air temperatures were maintained at 10/5 °C (day/night) in two cold chambers and at 30/20 °C in two warm chambers (± 1 °C). Photosynthetically active radiation (PAR) at leaf height in the chambers was maintained near $385 \mu\text{mol m}^{-2} \text{s}^{-1}$ with a 14 h photoperiod. A repeated measures ANOVA revealed no difference in measured PAR between cold and warm chambers throughout the experiment ($F_{1,2} = 0.001$, $P = 0.977$).

Within each chamber, each population was represented by up to six genetic lines (Table 1) with up to three replicates per line (depending on seed supply). In total the four chambers contained 200, 200, 202, and 198 pots in random order. Three seeds were sown in 164 ml "cone-tainers" (Stuewe and Sons, Inc., Tangent, OR, USA) filled with Pro-Mix HP potting soil. The pots were thinned daily such that only the first emerging seedling was allowed to grow. The pots were bottom-watered to maintain non-limiting soil moisture, such that treatment effects could be attributed to temperature differences and not any covarying water stress.

Emergence and growth

Pots were checked daily and emergence was recorded. After emergence and thinning, a total of 762 plants were grown throughout the course of the experiment. To examine early growth and morphology, the number of leaves and length of the longest leaf was measured for all plants at 2 weeks post emergence (specific to each plant). Plants were harvested at 55 days post emergence and aboveground tissue was separated, dried at 60 °C for 1 week, and weighed. Roots were rinsed, dried, and weighed for a subset of plants (98 total).

Physiological measurements

We selected a subset of plants (based on logistical constraints) to be used for measurements of leaf-level photosynthetic gas exchange and freezing tolerance (assessed by chlorophyll *a* fluorescence from Photosystem II). We selected a pair of cold- and warm-grown plants from up to three genetic lines per

population for physiological measurements (78 total). Each plant was selected at random, provided that seedlings had emerged within the first 5 days of emergence (two plants could not meet this criteria and were therefore slightly younger).

Leaf-level maximum photosynthetic rate (A_{max}) was assessed across a range of leaf temperatures, averaging 6.5, 14.1, 25.2, and 36.6 °C (for all leaf temperatures, standard deviations <0.7 °C). Measurements were carried out over 4 days (beginning 38 days after seeds were sown), with all plants exposed to one leaf temperature each day. Leaf temperatures were manipulated by measuring plants inside of a fifth growth chamber set to a fixed temperature. Plant pairs (cold- and warm-grown) were measured in succession, with the order of pairs randomly chosen each day. Plant pairs were moved from their primary growth chambers into the measurement chamber at least 2 h prior to being measured. 75 % of plants were measured between 2 and 2.5 h of the leaf temperature treatment and 93 % within 3 h (the longest treatment time was 3.4 h). Photosynthetic rates were measured using a LI-6400 portable photosynthesis system (Li-Cor, Inc., Lincoln, NE, USA). We selected the second youngest leaf on each plant for measurements and continued to measure the same leaf at each temperature setting. PAR in the leaf chamber was maintained at 1,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which had been determined to be saturating from preliminary tests. We maintained the CO_2 concentration in the leaf chamber at 380 $\mu\text{mol mol}^{-1}$ and kept relative humidity at ambient conditions (average = 40 %). Leaf area (for determining gas fluxes) was determined by measuring the width of each leaf blade at its midpoint in the rectangular leaf chamber (3 cm in length). Values were calculated as the average of 6 measurements logged at 5 s intervals following photosynthetic stabilization.

The same plants used for gas exchange measurements were also evaluated for freezing tolerance just before they were harvested at 55 days of growth. Entire plants in their cone-tainers were placed in a freezer overnight, and the temperature was gradually reduced from room temperature to -15 °C over a 3 h period. Freezing conditions were maintained between -10 and -15 °C for 8 h, at which point the freezer was shut off and the temperature was allowed to rise to above 0 °C over 2 h. Plants were then removed from the freezer and allowed to thaw in the dark at room temperature for another 90 min.

Leaf freezing tolerance was estimated as the decrease in F_v/F_m following freezing. F_v/F_m is determined by measurements of chlorophyll *a* fluorescence from Photosystem II (PSII) and represents the fraction of absorbed PAR that is able to be used for photochemistry in a dark-adapted leaf (Lambers et al. 1998). Values of F_v/F_m are proportional to quantum yield and reflect the overall photosynthetic capacity of PSII. We measured F_v/F_m before and after freezing for dark-adapted leaves using a Li-Cor LI-6400-40 LCF chamber. Inside the leaf chamber, leaves were exposed to a 0.8 s saturating flash ($\sim 6,000 \mu\text{mol m}^{-2} \text{s}^{-1}$) to induce maximal fluorescence (F_m). Plant pairs were measured in random order.

Statistical analyses

We tested for differences in the plastic response to temperature using a general linear model. As our experimental design was not conducive to survival analysis for emergence rates, we chose the median emergence time (i.e. number of days to 50 % emergence) and the final percent emergence per genetic line as response variables. Values of longest leaf, shoot biomass, root biomass, and median emergence time were log-transformed prior to analyses to satisfy assumptions of normality. Values of percent emergence were arcsine-square root transformed. Freezing tolerance was represented as a single response variable by taking the difference in F_v/F_m before and after freezing. These values were then log-transformed to satisfy the assumption of normality: $\ln(1 + F_v/F_m^{\text{post}} - F_v/F_m^{\text{pre}})$. Thus, more negative values represent lower freezing tolerance, with zero indicating no detected effect of the freezing treatment.

The use of growth chambers, while often necessary for particular experiments, can complicate and limit statistical analyses (Potvin 2001). We monitored chamber conditions throughout the experiment and never found any indication of environmental differences (temperature, RH, PAR) between chambers of the same temperature treatment. Furthermore, initial analyses using the full dataset indicated that there were few differences in response variables associated with a chamber effect, which was demonstrated by very small variance components (max. <4 %). We therefore simplified the analyses by calculating averages for genetic lines across chambers of the same temperature treatment. This does not inflate degrees of

freedom in our statistical tests as the denominator degrees of freedom for the effects of interest are based upon population and/or temperature treatment levels and not the overall residual (see below).

The main general linear model included the following fixed effects: source region (R), growth temperature (GT), and source region \times growth temperature (R \times GT). Random, nested effects were used in calculating fixed effects *F* ratios: population (P(R)) was used as the error term to test the effect of R, and growth temperature \times population (GT \times P(R)) was used as the error term to test the effects of GT and R \times GT. The overall residual of the model represented variation among genetic lines. Photosynthetic rates were analyzed using a repeated measures MANOVA and thus included the effect of measurement leaf temperature (LT). All statistical models were performed in JMP 7 (SAS Institute, Cary, NC, USA). All reported means and measures of variance were calculated hierarchically, e.g. population-level means are based on the means of genetic lines.

We also generated plasticity index (PI) values in order to examine plasticity as a univariate response (as opposed to an interaction effect). PI values for each genetic line were calculated as, $(a - b)/\text{Max}(|a|, |b|)$, where *a* and *b* are mean response values in warm and cold treatments, respectively (Valladares et al. in prep., after revision of estimates in Valladares et al. 2006). PI values are centered on zero (no plasticity) and are bound between -1 and 1 , with positive values indicating higher response values in the warm treatment and negative PI values indicating higher response values in the cold treatment. For example, a negative PI value for freezing tolerance indicates greater freezing tolerance for plants grown in the cold treatment. We examined relationships between plasticity and source environmental conditions using linear regressions of PI values and mean annual temperature, mean annual precipitation, and CI (Table 1). Relationships were initially examined separately for temperature and precipitation, and then with the CI if both were found to correlate with PI values.

Results

Emergence and growth

Initial emergence was most rapid in the warm temperature treatment although the emergence rate

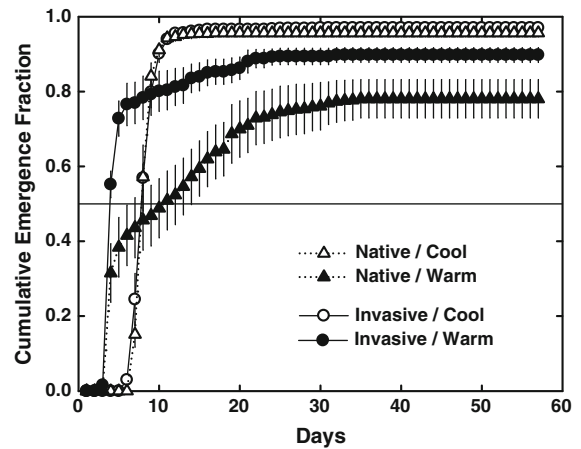


Fig. 1 Cumulative emergence for native and invasive *B. tectorum* populations across temperature treatments. The horizontal reference line indicates 50 % emergence. Values are population-level means \pm 1 SE

Table 2 Analysis of variance *P*-values for fixed-effect tests

| Measure | Source of variation | | |
|-----------------------|---------------------|---------|---------------|
| | R | GT | R \times GT |
| Median emergence time | 0.003* | 0.800 | 0.002* |
| Percent emergence | 0.034* | 0.001* | 0.262 |
| Leaf number | 0.478 | <0.001* | 0.386 |
| Longest leaf | 0.986 | <0.001* | 0.269 |
| Shoot biomass | 0.683 | 0.099 | 0.604 |
| Root biomass | 0.480 | 0.965 | 0.501 |
| Root:shoot | 0.142 | 0.237 | 0.762 |
| Freezing tolerance | 0.216 | 0.001* | 0.031* |

Main effects are represented as source region (R) and growth temperature (GT)

* Statistical significance ($P < 0.05$)

of native seeds quickly declined. Native seeds in the warm treatment required 13 days to reach 50 % cumulative emergence success compared to just 6 days for invasive seeds (Fig. 1). Final mean percent emergence was lowest in the warm treatment, particularly so for native seeds (78 %). Emergence in cold temperatures was slightly delayed, but then proceeded rapidly to near 100 % within a week, regardless of seed source region. Thus, emergence differences between native and invasive populations were only exhibited at warm temperatures, with invasive seeds emerging more rapidly than native seeds. This differential response of source region to temperature is

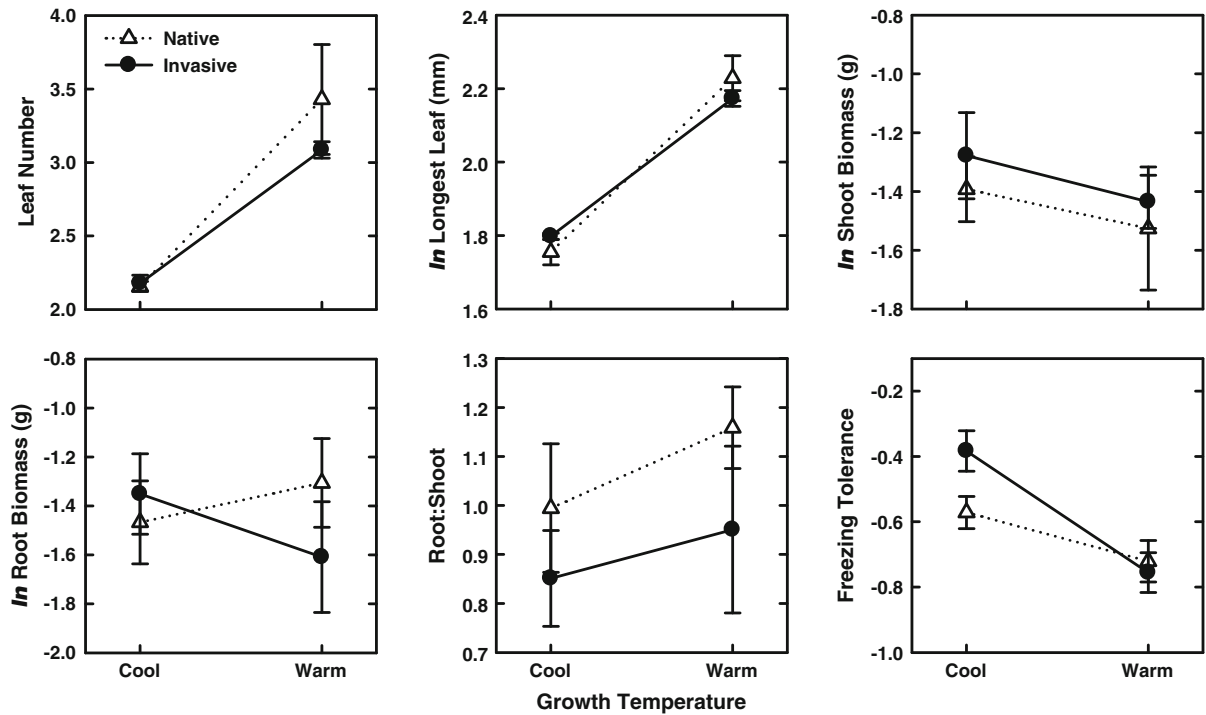


Fig. 2 Norms of reaction for native and invasive populations of *B. tectorum*. Leaf number and longest leaf length were measured 14 days after emergence, whereas biomass values and root:shoot were measured 55 days after emergence. Freezing tolerance is presented as the difference in chlorophyll

a fluorescence from Photosystem II before and after a simulated freezing event, $\ln(1 + F_v/F_m^{\text{post}} - F_v/F_m^{\text{pre}})$; higher values correspond to greater freezing tolerance. Values are population-level means ± 1 SE

indicated by a significant interaction effect in the ANOVA of median emergence time (Table 2), although this interaction was not significant for overall percent emergence.

Two weeks after emergence, plants grown in warm temperatures exhibited significantly more and longer leaves (Fig. 2), but there was no interaction with source region (Table 2). After 55 days of growth, there were no significant effects of source region, growth temperature, or their interaction on shoot biomass, root biomass, or root:shoot (Fig. 2; Table 2).

Physiology

There was a strong effect of the freezing treatment on F_v/F_m values and thus the inferred maximum quantum yield. Overall mean F_v/F_m values decreased from 0.80 (indicative of efficient energy transfer through PSII) before freezing to 0.37 after freezing. Freezing tolerance (as measured by the decrease in F_v/F_m) was greatest for plants grown in cold temperatures (Fig. 2), consistent with an adaptive plastic response. This effect

was most pronounced in invasive populations as demonstrated by a significant interaction between growth temperature and source region (Table 2). In the cold treatment, freezing reduced mean F_v/F_m from 0.78 to 0.49 for invasive populations compared to a reduction from 0.78 to 0.36 for native populations. In the warm treatment, mean F_v/F_m values after freezing were 0.31 for both source regions.

Plants exhibited adaptive plasticity for maximum leaf-level photosynthetic rates (A_{max}), such that cold-grown plants outperformed warm-grown plants at cold leaf temperatures and warm-grown plants outperformed cold-grown plants at warm leaf temperatures (significant $GT \times LT$ interaction; Table 3; Fig. 3). Cold-grown plants achieved their highest values of A_{max} around 14 °C with no further increase at higher temperatures. By contrast, A_{max} for warm-grown plants continued to increase at higher leaf temperatures. However, there was no difference in this plastic response between source regions as indicated by the three-way interaction term (Table 3).

Table 3 Repeated measures analysis of variance P -values for maximal carbon assimilation (A_{max}) in *B. tectorum*

| Measure | Source of variation | | | | | | |
|-----------|---------------------|-------|---------|--------|--------|---------|-------------|
| | R | GT | LT | R × GT | R × LT | GT × LT | R × GT × LT |
| A_{max} | 0.378 | 0.493 | <0.001* | 0.545 | 0.201 | <0.001* | 0.289 |

Main effects are represented as source region (R), growth temperature (GT), and measurement leaf temperature (LT). All terms with LT represent within-subjects effects in the analysis

* Statistical significance ($P < 0.05$)

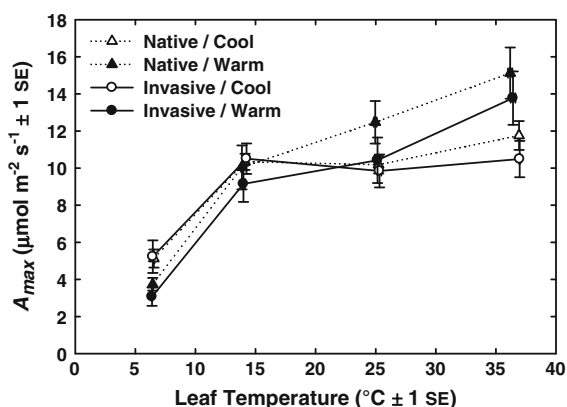


Fig. 3 Maximum photosynthetic rate (A_{max}) over a range of leaf temperatures for native and invasive *B. tectorum* populations grown at different temperatures. Values are population-level means ± 1 SE

Plasticity index values and source climate

Plasticity index values were related to source environmental conditions to examine whether variability in plasticity could be explained by local abiotic factors, and whether these relationships differed between native and invasive regions (important differences could exist in this regard even if *mean* levels of plasticity do not differ across regions). Response variables that showed overall differences in plasticity between source regions through analysis of reaction norms (emergence time and freezing tolerance) also differed in their mean PI values, as expected. For emergence time, native populations had positive PI values (mean = 0.218), whereas the direction of plasticity was the opposite for invasive populations (mean = -0.307; $F = 13.82$, $P = 0.003$). As a positive PI value indicates a relatively higher response value in the warm treatment, native populations are interpreted as emerging relatively slowly in the warm treatment (higher median emergence time in warm treatment), whereas invasive seeds emerged more

rapidly. PI values for median emergence time were related to both source temperature ($P = 0.046$, $r^2 = 0.58$) and precipitation ($P = 0.009$, $r^2 = 0.77$), but only for invasive populations. We therefore examined this relationship using the CI (Table 1) as an aggregate measurement of temperature and precipitation ($P = 0.017$, $r^2 = 0.71$ for invasive populations; Fig. 4). Thus, invasive populations from relatively cool/wet locations exhibited faster emergence when grown in warm temperatures, whereas those from hot/dry locations exhibited more variable plastic responses.

For freezing tolerance PI values, invasive populations (mean = -0.44) were more plastic than native populations (mean = -0.14; $F = 6.134$, $P = 0.029$). The direction of plasticity was generally consistent in that all but one population (Echmiadzin) exhibited greater freezing tolerance when grown in the cold treatment. Plasticity in freezing tolerance across both source regions (i.e. using all populations) was related to source mean annual temperature ($P = 0.010$, $r^2 = 0.44$; Fig. 4) and to source elevation ($P = 0.004$, $r^2 = 0.50$; data not shown), such that plants from colder source locations exhibited greater freezing tolerance when grown in cold conditions, regardless of invasive status. However, these relationships were not significant when examined within each region separately.

Discussion

This study compares variation in phenotypic plasticity in response to temperature for populations of *B. tectorum* in its native range and along an invasive front. The focus here is on the ecological context of plasticity and its relevance to population success in novel environments. Thus, this study attempts to standardize environmental conditions across native and invasive locations and asks whether invasive

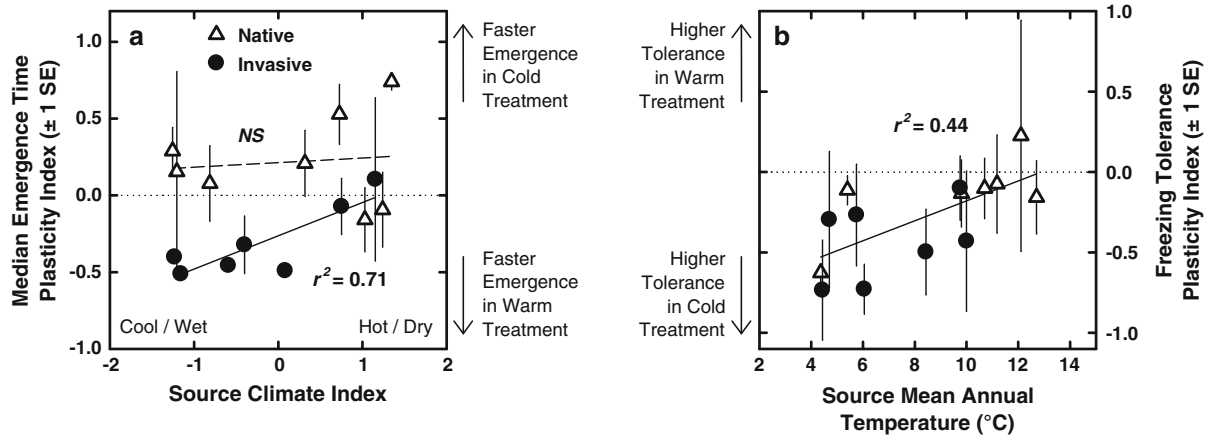


Fig. 4 Regression of *B. tectorum* plasticity index (PI) values against population source climatic variables for median emergence time and freezing tolerance. Emergence time PI values are examined separately for each region, whereas freezing tolerance PI is related to source mean annual

temperature for native and invasive populations combined. A lack of plasticity is indicated by the reference line at PI = 0, with increasing plastic responses as values become more positive or negative

populations of *B. tectorum* may rely on plasticity to invade semi-arid shrub-steppe ecosystems compared to native populations that may be more locally adapted.

Overall, we found significant differences in the degree of plasticity between native and invasive populations for important traits: invasive plants exhibited rapid germination in response to warm temperatures as well as greater freezing tolerance when grown in cold conditions. Additionally, both native and invasive populations together exhibited a cline in freezing tolerance plasticity related to source location temperature. In contrast, there were little differences in plasticity for growth-related traits between native and invasive populations. However, some growth related-traits, such as leaf number, leaf length, and maximal carbon assimilation, were strongly plastic across all populations. This suggests that plasticity in some important traits may be variable across populations, whereas other traits may be plastic for the species as a whole. However, the results in this case are more interesting and complex when discussed within the context of their specific functional and ecological relevance rather than in attempt to generalize in a broad manner.

Emergence

One of the most striking differences in plasticity between native and invasive populations was seen in

rates of seedling emergence. Emergence was strongly temperature-dependent, with relatively rapid emergence (and higher total emergence) for invasive populations, but only when grown at warm temperatures (Fig. 1). In cold temperatures, emergence reached nearly 100 % in 10 days for all seeds. When looking at the median emergence time, plasticity in native and invasive populations was divergent, with faster emergence in warm conditions for invasive populations and the opposite for native populations (Fig. 1). However, after achieving about 75 % emergence, cumulative emergence rates were lower in the warm temperature for both source regions (Fig. 1). Had we allowed all of the emerging seedlings to continue growing ($n = 1,990$ seeds), invasive plants in the warm treatment would have been on average 2.2 days older than native, warm-grown plants, and roughly 4 days older than all cold-grown plants. As an annual, seemingly small differences in age may have ecological consequences for *B. tectorum*, especially in locations where emergence is restricted until the spring and the growing season shortened. However, this may also be countered demographically by differences in total percent emergence between warm and cold conditions.

Although rapid emergence has been linked with invasive success (Schlaepfer et al. 2010; O'Donnell and Pigliucci 2010; Goergen and Daehler 2001), the broader implications of rapid emergence for *B. tectorum* are likely dependent on other environmental

factors, such that in some cases it is highly undesirable. Population growth of *B. tectorum* is strongly sensitive to rates of seedling germination and establishment, more so than to any other life history transition (Griffith 2010). Thus, the timing of emergence for this annual grass may be absolutely critical for invasive success, and can represent a strong mortality filter. Emerging too early may be detrimental for high elevation populations (Griffith and Loik 2010) or in years with particularly harsh winters (Mack and Pyke 1983). Seeds from the Mojave Desert have been observed to be conservative in their germination, potentially to avoid early germination triggered by episodic summer rain (Meyer et al. 1997). At the same time, plants that are able to emerge early and survive can subsequently achieve high rates of fecundity in the following spring through longer periods of active growth (Mack and Pyke 1983).

Beyond overall mean differences, we observed that the degree of plasticity for median emergence time was related to source climate for invasive populations, but not for natives (Fig. 4). Invasive populations from cool/wet locations emerged rapidly in response to warm temperatures, whereas those from warm/dry locations were more variable in their temperature response, but with average PI values near zero (i.e. variable plastic responses within populations canceled each other out). In particular, the two invasive populations from the hottest/driest sites (Carson and Benton) are the most geographically separated, and yet displayed similar responses related to their similar source climatic conditions. Ecologically, these responses may be advantageous in their respective environments and are generally consistent with prior results (Meyer et al. 1997) in which *B. tectorum* seeds from montane locations in western North America were some of the least conservative in terms of germination.

The adaptive significance of these responses is complicated, in that germination and emergence timing for *B. tectorum* is determined by patterns of seed after-ripening, secondary dormancy induction, and degree day accumulation (Beckstead et al. 1996; Roundy et al. 2007)—responses that can be influenced by genetics and the environment. In this case, the seeds were all exposed to the same conditions prior to emergence. However, our results are consistent with other evidence for germination- and emergence-related differentiation in invasive populations of *B.*

tectorum (Ramakrishnan et al. 2006; Meyer et al. 1997; Kao et al. 2008). Furthermore, it is interesting to observe population differentiation, not just in trait values, but in the degree of plasticity as a trait in and of itself.

Freezing tolerance

Both native and invasive populations exhibited adaptive plasticity in response to freezing, such that freezing tolerance was greater for cold-grown plants. Notably, this plastic response was greatest for invasive populations on average. Similar to plasticity in emergence timing, freezing tolerance plasticity was also related to source climate (Fig. 4), but across both source regions combined. However, invasive populations included more high-elevation and colder sites, so the difference between native and invasive populations may also reflect relative positions on an environmental cline in freezing tolerance plasticity for all populations. Regardless, it is notable that invasive populations seem to exhibit a degree of local adaptation for plasticity in freezing tolerance based on the observed cline. This is in contrast to invasive *Verbascum thapsis* populations in California, which are apparently mismatched with environmental conditions, such that some of the most freeze-tolerant individuals are located at lower elevations (Parker et al. 2003).

With a few exceptions, there is comparatively little research on the role of freezing tolerance in plant invasions. There are many possible angles to address this broad question, and several studies have demonstrated either superior freezing tolerance and/or population differentiation for invasive plants. For example, McEwan et al. (2009) found that the invasive shrub, *Lonicera maackii*, easily tolerated a late spring freeze while native shrub competitors suffered substantial leaf mortality. Invasive *Tamarix* species, on the other hand, had much lower freezing tolerance than native *Populus deltoides*, but exhibited a clear latitudinal cline in freezing tolerance and winter survival across its invasive range (Friedman et al. 2008). To our knowledge, ours is the first study to compare freezing tolerance and its plasticity in native and invasive populations of the same species.

Like seedling emergence, it is difficult to generalize upon the implications of plasticity in freezing tolerance with regard to the overall invasion of *B. tectorum*. The ability to tolerate freezing events is likely

important both in much of its home range in Eurasia as it is in its invasive range in North America. However, increased freezing tolerance and plasticity may encourage range expansion into high latitude/elevation sites, and/or increase the probability of establishment for low-density founder populations. In this study, the mean plastic response of invasive plants was nontrivial (a 39 % decrease in F_v/F_m for cold-grown plants compared to a 61 % decrease for warm-grown plants), suggesting that phenotypic plasticity for freezing tolerance likely has ecological implications for invasive *B. tectorum*.

Morphology and growth

Both native and invasive populations were very plastic in terms of leaf number and length (Fig. 2) in an apparently adaptive manner, with cold-grown plants assuming a stout, almost rosette-like form, whereas warm-grown plants had long and flexible leaves. Similarly, all populations showed a photosynthetic response indicative of adaptive plasticity, such that cold-grown plants exhibited relatively higher rates of photosynthesis in the cold, but warm-grown plants had higher photosynthetic rates at higher temperatures (Fig. 3). However, there was no consistent response to temperature for measurements of biomass and allocation patterns, and no differences observed overall between native and invasive populations for these traits (Fig. 2, see Fig. S4 in Electronic Supplementary Material). Regardless of native status, *B. tectorum* plants displayed a growth ability in the cold comparable to the warm treatment in terms of biomass accumulation.

These responses could all be linked as plasticity in photosynthesis and aboveground morphology may help maintain relatively consistent biomass accumulation across a range of conditions. Thus, a plant may be very plastic in one sense (i.e. particular functional traits), but this contributes to an overall 'jack-of-all-trades' result with *less* variability across environments in terms of population-level consequences (Richards et al. 2006). For an annual species like *B. tectorum*, biomass is tightly linked to lifetime seed production (Griffith 2010), and plasticity in function that helps to maintain biomass accumulation is likely to be important.

Conclusions

We found evidence for greater plasticity in invasive populations of *B. tectorum* for seedling emergence timing and freezing tolerance, but not for growth-related traits such as biomass, allocation, aboveground morphology, and photosynthesis. Rather, some growth-related traits exhibited plasticity across all populations, which may help to dampen differences in biomass in contrasting environments. Thus invasive populations may be particularly plastic for some important traits, but plasticity at the species level may also be an important factor in invasive ability. Irrespective of the comparison to native populations, the invasive populations in this study exhibited responses that indicate an ability to tolerate cold temperatures through flexibility in phenotypic expression. This may be an important factor in the ability of invasive *B. tectorum* to maintain and/or expand its invasive front into higher elevations.

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