Germination traits largely do not evolve post-invasion: Comparisons in the native and introduced range of seven herbaceous plant species

Harold N. Eyster[1,2,3] and Elizabeth M. Wolkovich[2,3,4]

[1]*Institute for Resources, Environment, and Sustainability, University of British Columbia, 429-2202 Main Mall, Vancouver, BC, Canada V6T 1Z4*

[2]*Arnold Arboretum of Harvard University, 1300 Centre Street, Boston, MA 02130, USA*

[3]*Department of Organismic & Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138,USA*

[4]*Department of Forest and Conservation Science, University of British Columbia, 3041-2424 Main Mall, Vancouver, BC, Canada V6T 1Z4*

Abstract

Invasive plants often have large impacts on ecosystems. Yet we lack a clear understanding of how some species become successful invaders while others do not. Two competing mechanisms have been posited: 1) post-introduction rapid evolution and 2) broad environmental tolerance in the source population. Discovering the determinants of invasion success requires more information on how these two mechanisms drive essential invasion traits, including germination rate, germination timing, and growth rate. Here, we tested for evidence of rapid evolution in these traits by using growth chambers to provide common environments for seven herbaceous plant species sampled from

multiple populations in their native (European) and invasive (North American) ranges. Chambers provided two levels of stratification—to simulate different winter lengths—and four temperature levels post-stratification—to simulate different spring conditions. Bayesian multilevel models enabled us to examine responses for each species, as well as across the suite of all seven species, while controlling for population and seed family effects. We found consistent results across all species: germination rate, germination timing, and growth rate were largely similar across native and invasive populations, except in response to particular combinations of high-temperature and stratification, generally representing cold winters and warm springs. Thus, we found little evidence of post-invasion evolution, except in response to specific multivariate climate. Overall, our results suggest that broad environmental tolerance likely underlies invasion success for this suite of common invaders.

Keywords: Invasion ecology, Rapid evolution, Broad environmental tolerance, Generalist, Phenology, Plant-climate interactions, Growth chamber experiment, Germination, Bayesian multilevel models, Invasive plants.

1 Introduction

Exotic plant invasions can transform biodiversity and ecosystems (Bellard et al., 2016; Mack et al., 2000; Pejchar & Mooney, 2009). These invasions are likely increasing: globalization is facilitating extra-range plant dispersal (Helmus et al., 2014), and human alteration of ecosystems may provide new niche space (Blois et al., 2013; Harte et al., 2015; Inouye, 2008; Tilman & Lehman, 2001). Upon dispersing to a new environment, invasive species can thrive by filling vacant niches (Elton, 1958) or outperforming native plants in high-resource and variable environments (Daehler, 2003; Davis & Pelsor, 2001). Changing environments, especially with anthropogenic climate change, could select for species that can take advantage of these newly created temporal niches and resources through shifts in the timing of flowering, fruiting, and other life history events. (Franks et al., 2007).

Thus, understanding the underlying drivers of plant invasion is becoming increasingly important. This understanding requires first identifying the biological mechanisms that plants may use to exploit invasible environments. Two contrasting mechanisms have been posited: 1) post-introduction rapid evolution and 2) broad environmental tolerance in the source population.

A large body of literature suggests that post-introduction rapid evolution is a key driver of invasion success (e.g., Clements & Ditommaso, 2011; Colautti & Lau, 2015; Lee, 2002; Prentis et al., 2008; Reznick & Ghalambor, 2001). Rapid evolution can enable nonindigenous species to adapt to vacant niches and take advantage of variable and high-resource environments, for example by evolving greater competitive ability when released from natural enemies (Blossey & Notzold, 1995; Bossdorf et al., 2005) or by evolving adaptive plasticity (Richards et al., 2006).

There are many clear examples of post-introduction rapid evolution abetting plant invasions. Genetic studies of two North American herbaceous goldenrods that invaded Europe, *Solidago altissima* and *S. gigantea* (Asteraceae), showed post-introduction genetic changes in flowering time in response to spring temperature, due to selection on source-population genetic variation and development of new mutations (Weber & Schmid, 1998). In California, a similar study found that genetic adaptation drove adaptive phenotypic variation in flowering time between high-altitude and desert populations of *Capsella bursa-pastoris* (Brassicaceae) (Linde et al., 2001). Invasion may even produce evolution sufficient to establish reproductive isolation and trigger speciation, in as few as 13 generations (Hendry et al., 2000). If post-introduction rapid evolution is this central to invader success, it would have important implications for invasive species management: managers should treat invasives not as static, homogeneous species, but as constantly adapting populations (Lee, 2002).

Yet, despite the support for the importance of post-introduction rapid evolution, a competing body of literature suggests that invaders need not evolve. Instead, broad environmen-

tal tolerance, plasticity and generalist adaptations to human-dominated environments (i.e., weediness) within the source population may give invaders sufficient advantages, obviating the necessity of post-introduction rapid evolution (Baker, 1965; Bock et al., 2015; Rejmanek & Richardson, 1996; Richards et al., 2006; Schwartz, 1994). Studies have found contrasting results regarding whether weediness in the native range is the best predictor of invasiveness (e.g., Maillet & Lopez-Garcia, 2000), or not (Mack, 1996; Perrins et al., 1992). Seeking a unified answer, a meta-analysis of 117 studies found that invasive plants were associated with performance-related traits, and concluded that it may be possible to predict future invaders by those traits (van Kleunen et al., 2010). In contrast to the rapid evolution hypothesis outlined above, this model of invasions would emphasize invasion prevention and, for invasions that cannot be prevented, treating them as a homogeneous population across their invasive range.

One reason why the importance of post-introduction rapid evolution remains contested is because few experimental designs allow discrimination between the two claims. Neither observational datasets (e.g., Wolkovich et al., 2013) nor simple common gardens (i.e., testing individuals from only one part of the range or in only one site, Conner & Hartl, 2004; Vitasse et al., 2009) are sufficient to discriminate. And while genetic studies can identify the existence of rapid evolution, they do not demonstrate the prevalence of this invasion mechanism. Reciprocal common garden experiments—with native and invader populations—can test these theories (e.g., Williams et al., 2008). For example, a reciprocal common garden experiment of two invasive maple species (*Acer*, Sapindaceae) demonstrated that rapid evolution was important for one species, but plasticity was important for the other (Lamarque et al., 2015). This and other studies show the promise of reciprocal common garden experiments for testing invasion mechanisms. Despite their utility, however, they are quite rare and typically only include one or two species due to the immense effort they require.

Growth chamber experiments offer an attractive alternative to reciprocal common gardens: they are easier to control and execute, thereby enabling a larger number of species to be

tested and compared simultaneously. Moreover, growth chambers can precisely vary the environments that plants experience and provide high-resolution assessment of small differences in trait responses. Testing such a multitude of species with growth chambers could help identify the mechanism(s) important for invasion success.

Another reason why the importance of post-introduction rapid evolution versus broad environmental tolerance is still contested may be because different traits may be controlled by different mechanisms. Some traits, such as flowering time, may be highly precise and under rapid selection (Weber & Schmid, 1998), while others may be broadly tolerant and stable. This is likely partly because some traits are known to evolve faster than others (Weiss-Lehman et al., 2017). The importance of post-introduction rapid evolution vs. broad environmental tolerance should thus be considered for specific traits, rather than overall effects. Therefore, understanding invasions requires understanding the mechanisms driving traits most essential for invasion.

Germination and growth traits are some of the most important for granting invasive success (Maillet & Lopez-Garcia, 2000; Sattin & Sartorato, 1997): invasive success requires the capacity to germinate in novel environments and grow rapidly enough to compete with native flora (Gioria & Pyšek, 2017; Grime et al., 1988). Therefore, germination rate (whether a seed germinates), germination timing (days between exposure to warm temperature and germination), and growth rate (cm/day) may represent key invasion traits. At least some of these traits appear to be sensitive to environmental differences (Leger & Rice, 2007). In particular they should respond strongly to two major germination cues: stratification length and spring temperature (Finch-Savage & Leubner-Metzger, 2006). In temperate ecosystems, many species require cold stratification, which simulates winter, before their seeds can germinate, a requirements that helps ensures that seeds do not germinate during a mid-winter warm period (Baskin & Baskin, 1998; Popay & Roberts, 1970; Wulff et al., 1994). Not surprisingly then, winter length is a key niche variable (Harte et al., 2015) that may show substantial spatial variation, independent of other climate variables (Bonan, 2003). Given

sufficient stratification length, spring temperature dictates the appropriate time for growth, and also plays an important role in controlling growth rate (Egli & Wardlaw, 1980; Guilioni et al., 2003).

Testing how germination and growth traits of invasive and native populations of multiple species respond to the environment (e.g., via stratification length and post-stratification temperature) could help illuminate whether rapid evolution versus broad environmental tolerance drives invasion. Here, we report on a growth chamber experiment comparing germination and growth traits of seven highly invasive herbaceous plant species collected from their native (Europe) and introduced (North America) ranges, many of which appear responsive to climate (Wolkovich & Cleland, 2014). Specifically, we measured how germination rate, time to germination, and growth rate of invasive (American) and native (European) conspecific populations responded to a full-factorial design of two stratification lengths and four spring temperatures. If post-introduction rapid evolution is of generalizable importance to invasive plants, we expect to find that seeds from the invading populations (North America) will respond very differently to spring temperature and stratification treatments than the native populations (Europe) for all or nearly all species.

2 Materials and Methods

2.1 Study species

Following Richardson's definition of invasive species (Richardson et al., 2011; Richardson et al., 2000, see Supp. for details), seeds were collected from eight herbaceous species that originated in Europe but were recently introduced to the US, where they have spread and produced substantial populations (Uva et al., 1997): *Alliaria petiolata*, *Capsella bursa-pastoris*, *Chelidonium majus*, *Dactylis glomerata*, *Plantago lanceolata*, *P. major*, *Rumex crispus*, and *Taraxacum officinale* (see Haines et al. (2011) for authorities). *Alliaria petiolata* exhibited

minimal germination, and so was removed from the analysis. These species represent a mix of perennials, biennials, and annuals. Many were intentionally introduced for medicinal or forage uses (for additional details, see Supp.). All of these species are invasive in the US, with many impacting crop production and transforming ecosystems (e.g., Froese & Acker, 2003; Wolfe et al., 2008).

Several of our study species (*Capsella bursa-pastoris, Chelidonium majus, Plantago lanceolata*, and *Rumex crispus*) are included in a phenology monitoring dataset (the Concord Phenology Dataset, Willis et al., 2008). This dataset shows that these species are on average flowering 4.5 days earlier than they did in the 1800s (compared to less than a day earlier for all 372 species in the dataset). This suggest that these invasive species exhibit flexible phenologies—flexibility that may be key to their success. Although this paper focuses on a different set of phenologies, the flexible flowering phenology suggests that these species may also exhibit flexible germination or growth rate traits. If so, this work can help determine if this flexibility may be due to rapid evolution. Thus, these species offer apt subjects to test the importance of post-introduction rapid evolution in invader germination and growth traits.

2.2 Seed collection

We collected mature seeds from native European populations and invasive North American populations from 15 June to 5 September 2015. European seeds came from 63 individuals across 13 sites in nine European countries: Austria, Denmark, France, Germany, Liechtenstein, The Netherlands, Norway, Slovenia, and Switzerland. North American seeds came from 21 individuals across three sites in Massachusetts, USA: Harvard Forest LTER (Petersham) Arnold Arboretum at Harvard University (Boston), and Walden Pond (Concord) (see Figure 1). Multiple seeds were collected from each parent plant (seed family). Elevation ranged from 0–1202 m in Europe and 20–300 m in USA. Seeds were collected in paper envelopes and stored at standard room temperature until early September 2015, when they

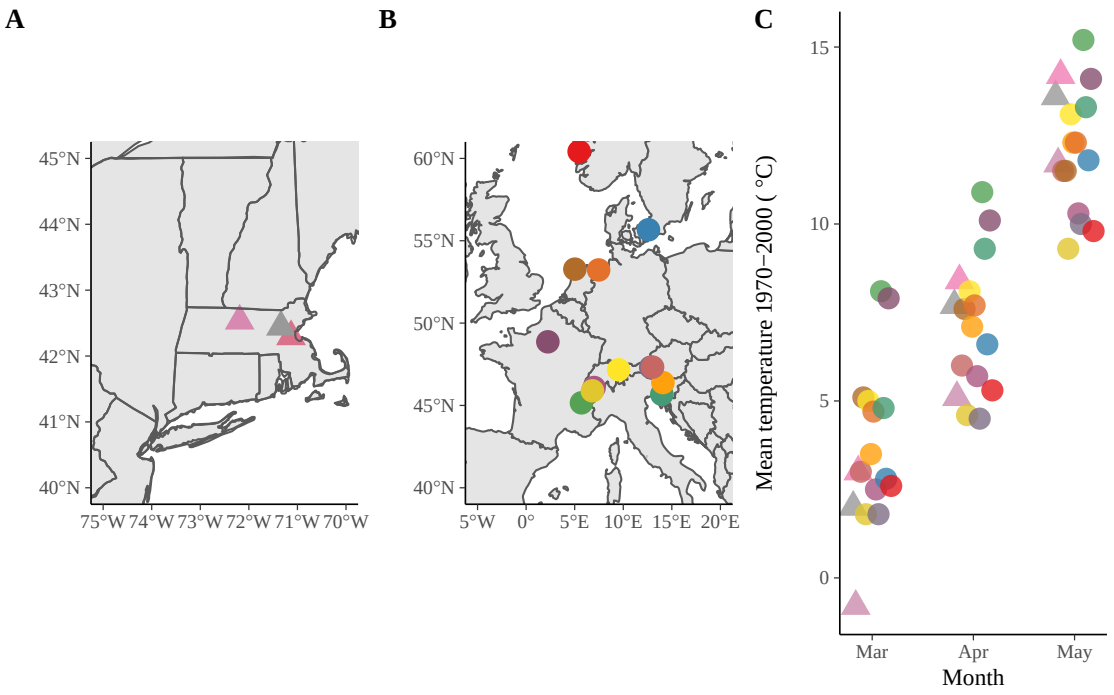

Figure 1: Map of collection sites of (A) invasive populations in New England and (B) native populations in Europe and (C) average March, April, and May temperatures at each site. Note that spring temperature at native populations (circles) are similar to spring temperature experienced by invasive populations (triangles), but also show key differences: invasive populations show colder March temperatures but warmer May temperatures.

were cleaned and returned to envelopes.

Climate: To examine how climate varied between populations and continents, the mean March, April, and May temperatures (~1 km^2 resolution) for 1970-2000 for each population location were downloaded from WorldClim Version 2 (Fick & Hijmans, 2017) and compared (see Figure 1). Climates were similar in the native/introduced populations, but still showed differences that may be sufficient to drive populations to adapt after invasion.

2.3 Experimental Design

To test phenological responses to climate, seeds were exposed to eight treatments representing varying climates. Seeds were first subjected to either a long or short stratification treatment, and then planted in one of four spring temperature treatments. All treatments were carried

out in growth chambers. For each treatment, 20 representatives of each species (with seven invasive species this equals 140 seeds per treatment) and an additional five representatives of each local population of *Plantago lanceolata* (the most heavily sampled species, with 13 populations) leading to a total of 205 seeds per treatment. Germination, time to germination, and aboveground linear height were recorded. Local population representatives were drawn from the greatest diversity of seed families, and seed family representation was equal across treatments.

2.4 Stratification

We stratified all seeds at 4°C, 70% humidity, 380 ppm of CO_2 (e.g., Meekins & McCarthy, 1999; Popay & Roberts, 1970) on moistened Whatman 1 qualitative filter paper in sterile, vented, light-version Greiner bio-one 94x16 petri dishes in darkness (Baskin & Baskin, 1998; Popay & Roberts, 1970) in a single Biochambers TPC-19 Reach-In Growth Chamber for either 30 days (reference level) or 60 days. These two stratification treatments represent intermediate stratification lengths for our species: studies show that our species require stratification lengths between 16 days (Popay & Roberts, 1970) and 120 days (Meekins & McCarthy, 1999). We began the 60-day stratification treatment in late September 2015; other seeds remained in paper envelopes at room temperature until they were in turn stratified in late October 2015. Water was added to petri dishes every 30 days.

2.5 Germination

On November 23, 2015, seeds from both stratification treatments were transferred into individual pots with soil (see Experimental Design, above), which were placed into four different growth chambers (three Biochambers TPC-19 and one Biochambers LTCB-19 Reach-In Growth Chamber) and subjected to four different germination treatments. Temperature varied across treatments—all other measured variables were kept constant, and treatments were rotated through growth chambers to control for unmeasured chamber effects. (Seeds

that germinated during stratification were not included in the analysis, but this was a small number and unlikely to affect results.)

Germination Temperature: Our four treatments used temperatures between 18 and 32°C. Optimal weed germination typically occurs at 20-30°C (Hartmann & Kester, 2010; Popay & Roberts, 1970; Steinbauer & Grigsby, 1957; Wulff et al., 1994). We used this sightly broader spectrum to ensure a sufficient variance in germination response.

Thermoperiocity: Our treatments employed daily fluctuations in temperature (thermoperiocity) of 10°C (see e.g., International Seed Testing Association, 1954; Steinbauer & Grigsby, 1957; Toole, 1963), translating to treatment temperatures of: 18/8°C (reference temperature), 22.67/12.67°C (temp1), 27.33/17.33°C (temp2), and 32/22°C (temp3). All treatments were subjected to 8 hours at the high temperature and the remaining 16 hours at the low temperature (Baskin & Baskin, 1998; Popay & Roberts, 1970; Probert, 2000; Roberts & Totterdell, 1981).

Light type, period, & luminance: We used T5HO fluorescent lights (Toole, 1963), which have a high R:FR ratio as, generally, exposure to a high R:FR ratio increases germination rates (though some studies find germination requires high R:FR ratio or is insensitive, Pons, 2000; Popay & Roberts, 1970; Wulff et al., 1994). We exposed all treatments to eight hours (coinciding with the higher temperature, Baskin & Baskin, 1998) of 75 micromol/m^2/second, which yielded a daily photon dosage of 2.16 mol/m^2. This amount of light should be sufficient to evoke germination response in all species (Pons, 1991). Because none of our species are known to exhibit high-irradiance response and growth chambers provide less light than normal natural conditions, we erred on the side of high light (see Supp. for additional details).

Planting substrate & water: We planted each seed in its own tray cell, on top of Fafard Growing Mix (a mixture of fine peat moss, fine perlite, and vermiculite) soil. We planted

seeds on top of soil both to ensure light availability (Tester & Morris, 1987) and because some species germinate poorly on filter paper (Andrews & Burrows, 1974). Every two days, seeds were watered until all of the soil had become wet (Steinbauer & Grigsby, 1957); but not so much that a film of water covered the seeds (Association of Official Seed Analysts, 1960).

Germination and growth rate monitoring: Collection of germination and growth data was masked to population. Seeds were checked during the light period for germination every two days. Germination was defined as the growth of shoot or radical through the seed coat (Baskin & Baskin, 1998; Popay & Roberts, 1970). Germination date for each seed was recorded. Germination was monitored until 29 Jan 2016, for a total observation length of 67 days (this is longer than the typical two-week germination trials according to Baskin & Baskin, 1998; Wulff et al., 1994). Aboveground linear height of each seedling was measured five times: 7 Dec 2015, 15 Dec 2015, 21 Dec 2015, 4 Jan 2016, and 29 Jan 2016. On 1 Jan 2016, the plants were moved from the growth chambers to a greenhouse subject to the following conditions: natural photoperiod (approximately 10 hours of light/day), 20 to 25°C, and 65% humidity.

2.6 Statistical analysis

To test for evidence of post-introduction rapid evolution across seven species, while accounting for effects of population and seed families, we used a Bayesian multilevel modeling framework (Carpenter et al., 2017). This approach yielded estimated (fixed) effects that fully incorporate these multiple levels of variance to produce overall estimates both for each species and generalized across species.

Plant height was roughly linear with time (see Figure S1), so growth rate was defined as β in the linear model: $height = \alpha + \beta * day + error$. This growth rate was calculated for each seed that germinated. For all models (growth rate, germination rate, and germination

timing), stratification length, continental origin, and temperature were treated as binary fixed effects, with the full suite of 2- and 3-way interactions included. Europe, 18/8°C, and 30 days were reference levels for origin, stratification length, and temperature, respectively; temperature was recoded as three dummy binary factors, allowing non-linear responses to temperature. Seed family was treated as a random effect, nested within sampling population, nested within species (with random slopes and intercepts). Growth rate was modeled with a normal error distribution:

$$y_i = N(\mu_i, \sigma) \tag{1}$$

$$\begin{aligned}\mu_i =& \alpha + \beta_1 \times origin + \beta_2 \times strat \\ &+ \beta_3 \times temp1 + \beta_4 \times temp2 + \beta_5 \times temp3 \\ &+ \beta_6 \times origin \times strat + \beta_7 \times origin \times temp1 \\ &+ \beta_8 \times origin \times temp2 + \beta_9 \times origin \times temp3 \\ &+ \beta_{10} \times strat \times temp1 + \beta_{11} \times strat \times temp2 \\ &+ \beta_{12} \times strat \times temp3_+ \beta_{13} \times origin \times strat \times temp1 \\ &+ \beta_{14} \times origin \times strat \times temp2 + \beta_{15} \times origin \times strat \times temp3)\end{aligned} \tag{2}$$

Where the α (intercept) and β (slope) coefficients were all specified with the same normally-distributed nested random effects: seed family nested within sampling population, nested within species—$sp[pop[sfamily[i]]]$ (not shown above). Thus, for each γ in $[\alpha, \beta_1 : \beta_{15}]$:

$$\gamma_{sp[k]} = N(\mu_\gamma, \sigma_\gamma) \tag{3}$$

$$\gamma_{sp[pop[j]]} = N(\mu_{\gamma_{sp[k]}}, \sigma_{\gamma_{sp[k]}}) \tag{4}$$

$$\gamma_{sp[pop[sfamily[i]]]} = N(\mu_{\gamma_{sp[pop[j]]}}, \sigma_{\gamma_{sp[pop[j]]}}) \tag{5}$$

Where sp = species, indexed with k, pop = sampling population, indexed with j, $sfamily$ = seed family, indexed with i, and $strat$ = stratification. Germination rate was modeled similarly to growth rate, but using a binomial error distribution and logit link function, while germination timing was modeled with a Poisson error distribution and log link function.

All models were estimated using four chains, each with 2000 iterations (1000 devoted to warm-up), and wide priors. All models were built with Stan (Carpenter et al., 2017) using `rstanarm` version 2.17.4 (Goodrich et al., 2018) in R (R Development Core Team, 2015). Chain convergence was confirmed using the Gelman–Rubin statistic/$\hat{R}$ close to one (Gelman & Rubin, 1992). Model implementations were validated using simulated data; model fits were assessed using posterior predictive checks (Gelman et al., 2004).

Average predictive comparisons: The interactions of treatments (stratification and temperature) and random effects (species, population and seed family) make this model complex, and can make clear interpretations of parameter estimates difficult. To address this, we calculated average predictive comparisons (Gelman & Pardoe, 2007) for each stratification and temperature level. These estimates average over interaction terms and the full mixed (fixed and random) effects, to provide a single estimate per level that includes all modeled uncertainty. Additionally, unlike model output from Poisson and Binomial models, which are given in transformed units, average predictive comparisons yield estimates that are in the units of the dependent variable (but always positive) (Gelman & Pardoe, 2007) and thus allow comparisons across effects. We note that average predictive comparisons can be complicated to implement in many unbalanced designs; because our stratification and temperature variables are balanced and independent (i.e., every combination of input values is equally likely to co-occur), we calculated average predictive comparisons without any weighting requirement, thus simplifying the computation. See Supplement for equations and details.

3 Results

Germination rate: Germination rate was high: across all species, populations, and seed families, 76% of seeds germinated. Overall, germination rate was insensitive to stratification, temperature, or origin—95% credible intervals (henceforth, 'CrI') for all effects were clustered around zero (Figures 3, S2; Table S2). Regardless of the climatic conditions, they germinated at fairly constant, high rates. Seeds from the invasive and native ranges germinated at similar rates and responded similarly to treatments (see 'origin,' 'strat,' 'temp1,' 'temp2,' 'temp3,' 'origin × strat,' 'origin × temp1,' 'origin × temp2,' 'origin × temp3,' 'strat × temp1,' 'strat × temp2,' 'strat × temp3,' 'origin × strat × temp1,' 'origin × strat × temp2,' 'origin × strat × temp3' in Figure 3 and Table S2). Seeds from different local populations also germinated at similar rates (see Figure S5).

Germination timing: The mean time to germination across all species, populations, and seed families was 12.33 days. Overall, stratification and seed origin had no noticeable effect (see 'origin' and 'strat' in Figure 3 and Table S3). All species germinated slower at the lowest temperature, but germinated at similar, faster speeds at the three higher temperatures, showing that temperature response is non-linear (see 'temp1,' 'temp2,' and 'temp3' in Figures 3, S3; Table S3). However, *Plantago lanceolata* did show faster germination in response to med-low temperature × stratification interaction (see Figure S5). Moreover, all species showed a significant positive interaction effect of origin, stratification and the higher temperature (95% CrI: 1.05–2.9 days; see 'origin × strat × temp3' in Figure 3 and Table S3). That is, the invasive population germinated slower at the long stratification/highest temperature combination. Populations showed fairly homogeneous responses, though temperature × stratification interactions did show some inter-population variability (see Figure S5).

Growth rate: The mean growth rate was 1.2 mm/day. Overall, growth rate was the most sensitive to treatments, though it was still unaffected by population origin length or stratification *per se* (see 'origin' and 'strat' in Figures 3, S4; Table S4). Growth rate decreased at warmer temperatures for all species, but especially *Dactylis glomerata* (see 'temp1', 'temp2', and 'temp3' in Figure 3). This effect was larger for each higher temperature; this is in contrast to germination timing, where the decrease with temperature was more constant (see comparison in absolute change displayed in Figure 2). However, this decreased growth rate at high temperatures was not uniform across all treatments: for one of the higher temperatures (temp2) seeds stratified for 60 days and originating in North America (the invasive range) grew 0.74mm faster per day (95% CrI: 0.22–1.27) than those stratified for 30 days from Europe (see 'origin × strat × temp2' in Figure 3 and Table S4).

4 Discussion

This study leveraged the power of a multi-species growth chamber experiment of native and introduced populations to investigate the importance of post-introduction rapid evolution for invasive species' success. All seven highly invasive plant species responded similarly to climate. Across all species, we found only isolated support for the prevalence of post-introduction rapid evolution of key invasion traits—germination rate, timing, and growth rate. Instead, our results are consistent with the theory that these traits do not need to evolve for these species to invade: weediness, wide environmental tolerance, plasticity, or generalist traits in the source populations may instead provide sufficient capacity to exploit novel environments (Baker, 1965). Post-introduction rapid evolution may provide a helping hand, but—at least for these traits and these species—rapid evolution does not appear broadly essential for invasion success.

These findings are especially pronounced in germination rate, where all species germinated well and with scant sensitivity to climatic conditions, suggesting that the source populations

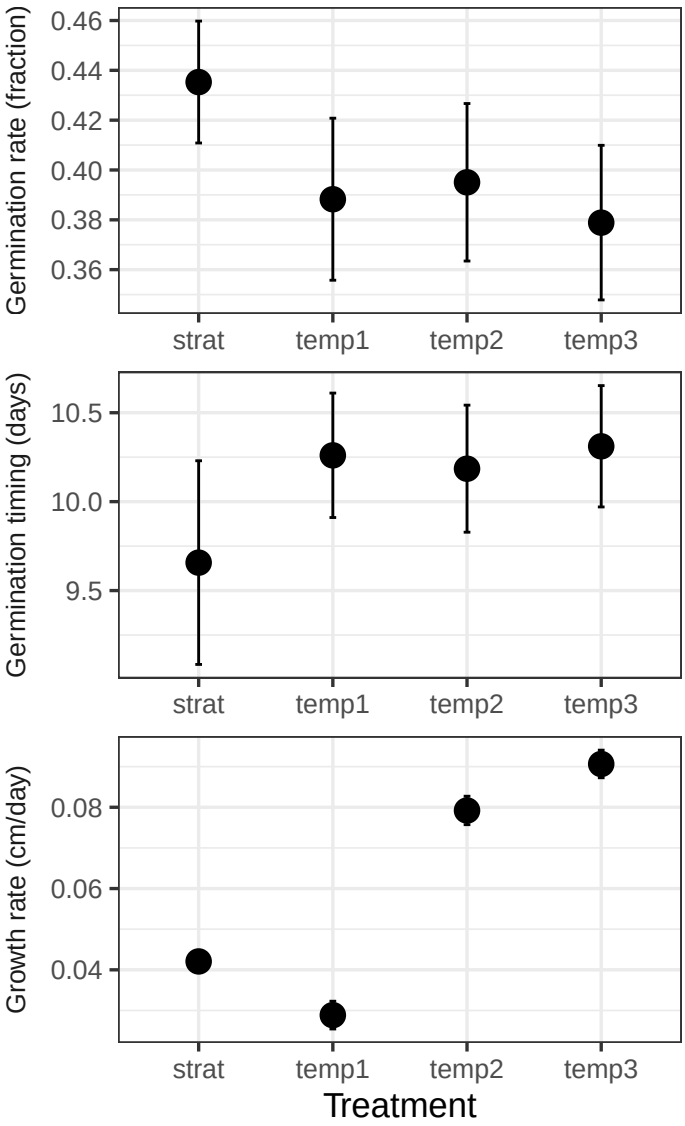

Figure 2: Average predictive comparisons of germination rate (top), germination timing (middle), and growth rate (bottom) show how much change in the dependent variable results from a one unit change in the predictor variable while at once integrating over uncertainty from other effects in the model. Higher temperatures had indistinguishable effects on germination timing (middle), but sequentially bigger effects on growth rate (bottom). See Supplement for further explanation.

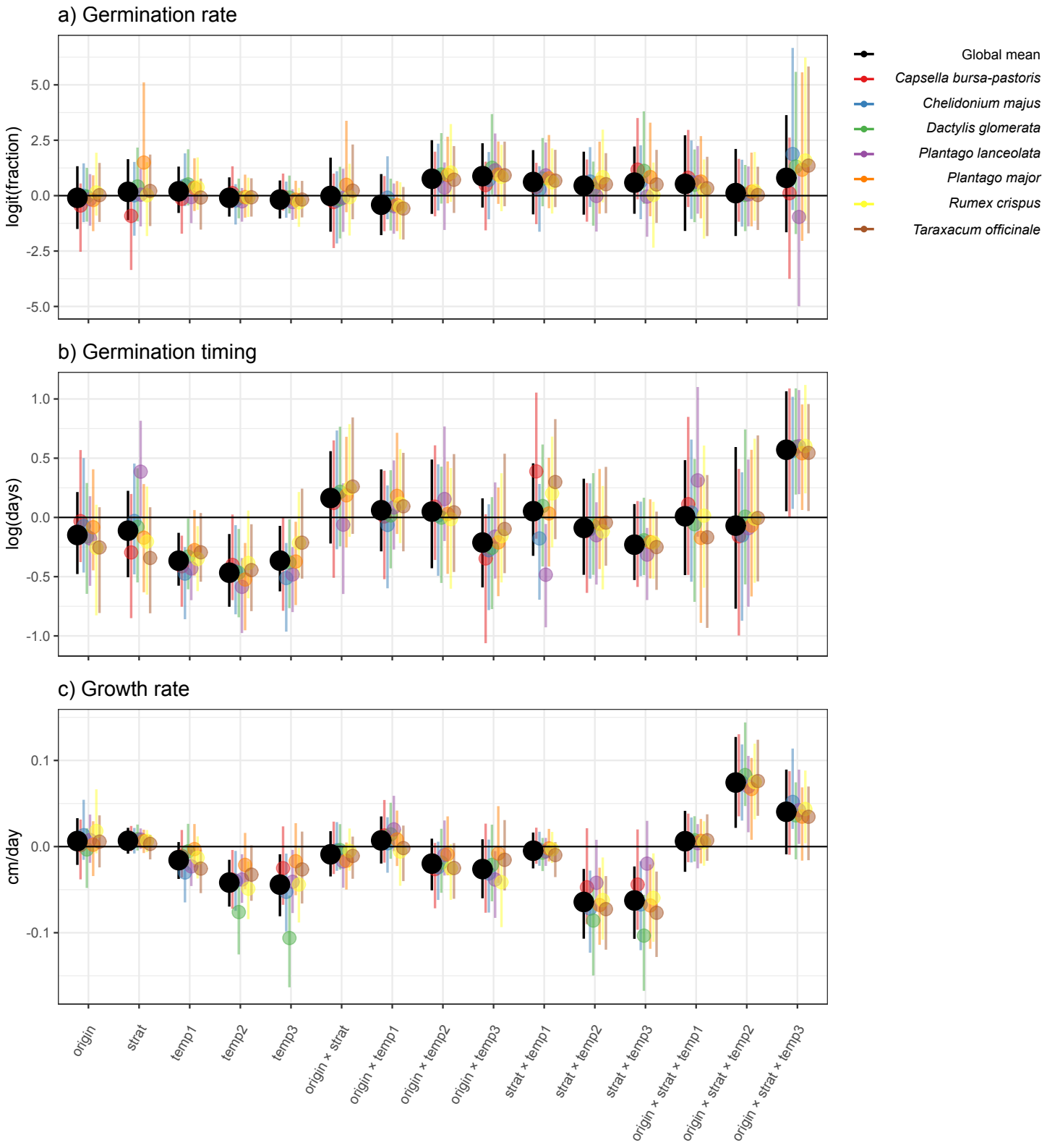

Figure 3: Multilevel model coefficients with 95% credible intervals for a) germination rate, b) germination timing and c) growth rate, showing global average effects and species random effects (intercept coefficients provided in the Supp. in Tables S2,S3, S4). The reference level for temperature is 18/8°C, while temp1 = 22.67/12.67°C, temp2 = 27.33/17.33°C, and temp3 = 32/22°C. Thirty days (30 d) is the reference level for stratification (thus, strat=60 d).

of invading species provided invaders with the capacity to germinate in diverse environments. Some have suggested that, while initially species may not need to evolve, they may evolve once achieving a foot-hold (Lamarque et al., 2015). However, many of the study species (e.g., *Dactylis glomerata*) have occupied their invasive range for centuries, yet still show little sign of an evolving, or evolved, germination response.

Overall, germination timing and growth rate did not show signs of post-introduction evolution. However, there was some evidence that particular responses have evolved: North American (invasive) populations germinate later and grow faster under long stratification/high spring temperature combinations. Taking the climate of North American populations into account (Figure 1), this rapid post-introduction evolution of growth rate may be adaptive. North American populations experience climates with longer winter stratification (lower mean March temperatures) and hotter spring temperatures (higher mean May temperature). Thus, the capacity to grow faster after being exposed to a long stratification treatment and high temperatures may provide fitness advantages. Our experimental design's inclusion of multiple seeds per seed family suggests that these results are not due to maternal effects. However, it is possible that these differences could be residual founder effects (Shirk et al., 2014), a result of genetic drift (Eckert et al., 1996), or that germination rate is not a fitness trait. Nevertheless, the convergence with experienced climate suggests that this observed change in growth rate is a sign of adaptive post-introduction rapid evolution.

These results highlight the need to condition biological invasion mechanisms on specific invasion traits. We found that post-introduction rapid evolution played no role in germination rate, but may play a role in growth rate under certain treatment conditions. This suggests that research and theory aimed at identifying which traits are likely to rapidly evolve with invasion may yield more insights than testing for an overall mechanism of invasion that is consistent across traits. We found evidence that germination timing and growth rate traits were most likely to evolve in response to specific combinations of spring temperatures and winter length. This result suggests that considering the interdependent multivariate environ-

ment in the invasive range may be critical for predicting how traits evolve post-introduction. Not only can these trait evolution/environment relationships be useful for understanding invasions, they can also help delineate plant capacities to adapt to the multifaceted effects of anthropogenic climate change.

Our findings preliminarily suggest that these invasive species may be able to adapt to changing climates by shifting germination timing or growth rate. The evidence that species can adapt their growth rate under certain conditions suggests that invasive species may have the capacity to adapt to changing winter lengths and warming spring temperatures that are expected under anthropogenic climate change (IPCC, 2015). These results also echo the importance of designing experiments that vary both winter length and spring temperature in order to observe responses to climate change (e.g., Bernareggi et al., 2016).

Our results come from a limited number of individuals and populations collected from the invasive range (see Figure 1; Table S1), yet our sampling sites show substantial climate variation (Figure 1), highlighting potentially important climatic differences between Europe and North America that may shape invasions. While additional sampling across the invasive range would have yielded greater geographical inference to our findings, it may also have made the complex stratification by temperature responses harder to detect—if such responses, and their evolution, are dependent on specific multivariate climates. Based on our findings, we suggest sampling across distinct invasive range climates could help understand which traits evolve where post-introduction.

We harnessed the benefits of growth chambers to provide a common set of precisely controlled multivariate environments for seven species; however, the benefits of this design trade off with a lack of realism. In contrast to reciprocal field common garden experiments, which can integrate important factors (Blois et al., 2013; Germain et al., 2018), our approach lacked most biotic interactions and natural climatic variation. Yet our approach let us tease apart the multivariate nature of climate (stratification $\times$ temperature) and examine

evidence for post-introduction rapid evolution across a large range of introduced climates. We believe combining similar growth chamber designs with Bayesian modeling approaches, which integrate across multiple levels of variance (species, population, seed family), provides a tractable approach for other populations, other traits, and other combinations of climate factors (including precipitation). Such future small-scale growth chamber studies could enable robust meta-analyses capable of identifying the traits and climate responses for which post-introduction rapid evolution is, or is not, essential for invasion success, and may guide where best to invest the intensive resources required for reciprocal field common garden experiments.

Our results show that post-introduction rapid evolution of germination and growth traits is unlikely to be essential for all plant invasions. Instead, it seems that broad environmental tolerance is key to invasion success for these seven species. Post-introduction rapid evolution may still play a role, especially in more extreme or different environments. Linde et al. (2001) found that *Capsella bursa-pastoris* evolved to colonize high-altitude and desert environments in California. In contrast, when comparing between temperate populations of this same species, we found little sign of rapid evolution, suggesting the generalist traits contained in temperate source populations may be suitable as long as the introduced environment is not too different (Baker, 1965). Our findings provide support for the speculation by van Kleunen and colleagues (2010) that future invasions can be predicted by species' characteristics, but perhaps only specific traits, such as germination rate. This finding suggests that managers can perhaps best guard against future invasions by targeting weedy species and preventing them from dispersing beyond their native ranges.

Acknowledgments Thanks to E. Forrestel for mentorship to HNE. Thanks to J. Williams and H. Branch for fruitful discussions. Thanks also to D. Flynn, S. Gee, J. Samaha, and T. Savas for help transplanting, collecting, and measuring. Thanks to F. Rosin, K. Woodruff, and J. Gard for help with collection and growing logistics. Thanks to S. Fritz for help with

data input. Thank you to A. Acosta, D. Buonaiuto, C. Chamberlain, A. Delgado, A. Ettinger, N. Gilbert, C. Husic, and M. Rucinski for comments on earlier drafts of this paper. Thanks to J. Eyster, S. Stalhandske, and G. Barbone for assistance with and camaraderie during field sampling. This field work was supported by the Harvard College Undergraduate Research Fund and the Harvard University Center for the Environment Undergraduate Summer Research Fund.

Authors' contributions HNE and EMW conceived the study and designed the methods; HNE led the data collection, analysis, and writing, with assistance from EMW. HNE and EMW contributed critically to the drafts and gave final approval for publication.

Data, code R code, Stan code, and data will be deposited on the Knowledge Network for Biocomplexity (KNB) repository.

References

Andrews, C. J., & Burrows, V. D. (1974). Increasing winter survival of dormant seeds by a treatment inducing secondary dormancy. *Can J. Plant Sci*, *54*, 565–571.

Association of Official Seed Analysts. (1960). *Rules for testing seeds* (tech. rep.). Proc. Assoc. Off. Seed Anal.

Baker, H. (1965). Charateristics and modes of origin of weeds. In H. Baker & G. Stebbins (Eds.), *The genetics of colonizing species* (pp. 147–168). Academic Press.

Baskin, C. C., & Baskin, J. M. (1998). *Seeds: Ecology, biogeography, and evolution of dormancy and germination*. San Diego.

Bellard, C., Cassey, P., & Blackburn, T. M. (2016). Alien species as a driver of recent extinctions. *Biology letters*, *12*(2), 20150623.

Bernareggi, G., Carbognani, M., Mondoni, A., & Petraglia, A. (2016). Seed dormancy and germination changes of snowbed species under climate warming: the role of pre- and post-dispersal temperatures. *Annals of Botany*, *118*(3), 529–539.

436 Blois, J. L., Zarnetske, P. L., Fitzpatrick, M. C., & Finnegan, S. (2013). Climate change and
437 the past, present, and future of biotic interactions. *Science*, *341*(6145), 499–504.
438 Blossey, B., & Notzold, R. (1995). Evolution of Increased Competitive Ability in Invasive
439 Nonindigenous Plants: A Hypothesis. *The Journal of Ecology*, *83*(5), 887–889.
440 Bock, D. G., Caseys, C., Cousens, R. D., Hahn, M. A., Heredia, S. M., Hübner, S., Turner,
441 K. G., Whitney, K. D., & Rieseberg, L. H. (2015). What we still don't know about
442 invasion genetics. *Molecular Ecology*, *24*(9), 2277–2297.
443 Bonan, G. B. (2003). *Ecological climatology*. Cambridge University Press.
444 Bossdorf, O., Auge, H., Lafuma, L., Rogers, W. E., Siemann, E., & Prati, D. (2005). Phe-
445 notypic and genetic differentiation between native and introduced plant populations.
446 *Oecologia*, *144*(1), 1–11.
447 Carpenter, B., Gelman, A., Hoffman, M. D., Lee, D., Goodrich, B., Betancourt, M., Brubaker,
448 M., Guo, J., Li, P., & Riddell, A. (2017). Stan: A Probabilistic Programming Lan-
449 guage. *Journal of Statistical Software*, *76*(1).
450 Clements, D. R., & Ditommaso, A. (2011). Climate change and weed adaptation: Can evolu-
451 tion of invasive plants lead to greater range expansion than forecasted? *Weed Research*,
452 *51*(3), 227–240.
453 Colautti, R. I., & Lau, J. A. (2015). Contemporary evolution during invasion: Evidence
454 for differentiation, natural selection, and local adaptation. *Molecular Ecology*, *24*(9),
455 1999–2017.
456 Conner, J. K., & Hartl, D. L. (2004). *A primer of ecological genetics*. Sinauer Associates
457 Incorporated.
458 Daehler, C. C. (2003). Performance comparisons of co-occurring native and alien invasive
459 plants: Implications for conservation and restoration. *Annual Review of Ecology, Evo-*
460 *lution, and Systematics*, 183–211.
461 Davis, M. A., & Pelsor, M. (2001). Experimental support for a resource-based mechanistic
462 model of invasibility. *Ecology letters*, *4*(5), 421–428.

Eckert, C. G., Manicacci, D., & Barrett, S. C. H. (1996). Genetic drift and founder effect in native versus introduced populations of an invading plant, Lythrum salicaria (Lythraceae). *Evolution*, *50*(4), 1512–1519.

Egli, D. B., & Wardlaw, I. F. (1980). Temperature response of seed growth characteristics of soybeans. *Agronomy Journal*, *72*(3), 560–564.

Elton, C. S. (1958). *The ecology of invasions by animals and plants, 2000th ed.* University of Chicago Press.

Fick, S. E., & Hijmans, R. J. (2017). Worldclim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*.

Finch-Savage, W. E., & Leubner-Metzger, G. (2006). Seed dormancy and the control of germination. *New Phytologist*, *171*(3), 501–523.

Franks, S. J., Sim, S., & Weis, A. E. (2007). Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(4), 1278–1282.

Froese, N. T., & Acker, R. C. V. (2003). Distribution and interference of dandelion (taraxacum officinale) in spring canola. *Weed Sci*, *51*, 435–442.

Gelman, A., Carlin, J. B., Stern, H. S., & Rubin, D. B. (2004). *Bayesian Data Analysis*. Chapman; Hall.

Gelman, A., & Pardoe, I. (2007). Average predictive comparisons for models with nonlinearity, interactions, and variance components. *Sociological Methodology*, *37*(1), 23–51.

Gelman, A., & Rubin, D. B. . (1992). Inference from Iterative Simulation Using Multiple Sequences. *Statistical Science*, *7*, 457–511.

Germain, R. M., Mayfield, M. M., & Gilbert, B. (2018). The 'filtering' metaphor revisited: Competition and environment jointly structure invasibility and coexistence. *Biology Letters*, *14*(8).

Gioria, M., & Pyšek, P. (2017). Early bird catches the worm: Germination as a critical step in plant invasion. *Biological Invasions*, *19*(4), 1055–1080.

Goodrich, B., Gabry, J., Ali, I., & Brilleman, S. (2018). rstanarm: {Bayesian} applied regression modeling via {Stan}.
R package version 2.17.4.

Grime, J., Hodgson, J. G., & Hunt, R. (1988). *Comparative Plant Ecology: a Functional Approach to Common British Species*. Unwin Hyman.

Guilioni, L., Wéry, J., & Lecoeur, J. (2003). High temperature and water deficit may reduce seed number in field pea purely by decreasing plant growth rate. *Functional Plant Biology*, *30*(11), 1151–1164.

Haines, A., Farnsworth, E., & Morrison, G. (2011). *New england wild flower society's flora novae angliae: A manual for the identification of native and naturalized higher vascular plants of new england*. Yale University Press.

Harte, J., Saleska, S. R., & Levy, C. (2015). Convergent ecosystem responses to 23-year ambient and manipulated warming link advancing snowmelt and shrub encroachment to transient and long-term climate–soil carbon feedback. *Global change biology*, *21*(6), 2349–2356.

Hartmann, H. T., & Kester, D. E. (2010). *Plant propagation: Principles and practices*. Prentice-Hall.

Helmus, M. R., Mahler, D. L., & Losos, J. B. (2014). Island biogeography of the anthropocene. *Nature*, *513*, 543–546.

Hendry, A. P., Wenburg, J. K., Bentzen, P., Volk, E. C., & Quinn, T. P. (2000). Rapid evolution of reproductive isolation in the wild: Evidence from introduced salmon. *Science*, *290*(5491), 516–518.

Inouye, D. W. (2008). Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology*, *89*(2), 353–362.

International Seed Testing Association. (1954). International rules for seed testing. In *Proc. international seed testing assoc.*

IPCC. (2015). *Climate change 2014: Synthesis report* (tech. rep.). Intergovernmental Panel on Climate Change, Geneva.

Lamarque, L. J., Lortie, C. J., Porté, A. J., & Delzon, S. (2015). Genetic differentiation and phenotypic plasticity in life-history traits between native and introduced populations of invasive maple trees. *Biological Invasions*, *17*(4), 1109–1122.

Lee, C. E. (2002). Evolutionary genetics of invasive species. *Trends in Ecology and Evolution*, *17*(8), 386–391.

Leger, E. A., & Rice, K. J. (2007). Assessing the speed and predictability of local adaptation in invasive California poppies (Eschscholzia californica). *Journal of Evolutionary Biology*, *20*(3), 1090–1103.

Linde, M., Diel, S., & Neuffer, B. (2001). Flowering ecotypes of Capsella bursa-pastoris (L.) Medik. (Brassicaceae) analysed by a cosegregation of phenotypic characters (QTL) and molecular markers. *Annals of Botany*, *87*(1), 91–99.

Mack, R. N. (1996). Predicting the identity and fate of plant invaders: emergent and emerging approaches. *Biological Conservation*, *78*, 107–121.

Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M., & Bazzaz, F. A. (2000). Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications*, *10*(3), 689–710.

Maillet, J., & Lopez-Garcia, C. (2000). What criteria are relevant for predicting the invasive capacity of a new agricultural weed? The case of invasive American species in France. *Weed Research*, *40*(1), 11–26.

Meekins, J. F., & McCarthy, B. C. (1999). Competitive ability of alliaria petiolata (garlic mustard, brassicaceae), an invasive, nonindigenous forest herb. *International Journal of Plant Sciences*, *160*(4), 743–752.

Pejchar, L., & Mooney, H. A. (2009). Invasive species, ecosystem services and human well-being. *Trends in Ecology and Evolution*, *24*, 497–504.

Perrins, J., Williamson, M., & Fitter, A. (1992). Do annual weeds have predictable characters?. *Acta Oecologica – International Journal of Ecology*, *13*, 517–533.

Pons, T. L. (1991). Induction of dark-dormancy in seeds: Its importance for the seed bank in the soil. *Functional Ecology*, *5*, 669–675.

Pons, T. L. (2000). Seed responses to light. *Seeds: the ecology of regeneration in plant communities*, 237–260.

Popay, A. I., & Roberts, E. H. (1970). Factors involved in the dormancy and germination of capsella bursa-pastoris (l.) medik. and senecio vulgaris l. *The Journal of Ecology*, 103–122.

Prentis, P. J., Wilson, J. R., Dormontt, E. E., Richardson, D. M., & Lowe, A. J. (2008). Adaptive evolution in invasive species. *Trends in Plant Science*, *13*(6), 288–294.

Probert, R. J. (2000). The role of temperature in the regulation of seed dormancy and germination. *Seeds: the ecology of regeneration in plant communities*, *2*, 261–292.

R Development Core Team. (2015). *R: A language and environment for statistical computing* (tech. rep.). R Foundation for Statistical Computing, Vienna, Austria. URL.

Rejmanek, M., & Richardson, D. M. (1996). What Attributes Make Some Plant Species More Invasive? *Ecology*, *77*(6), 1655–1661.

Reznick, D. N., & Ghalambor, C. K. (2001). The population ecology of contemporary adaptations: What empirical studies reveal about the conditions that promote adaptive evolution. In A. P. Hendry & M. T. Kinnison (Eds.), *Microevolution rate, pattern, process*. Springer.

Richards, C. L., Bossdorf, O., Muth, N. Z., Gurevitch, J., & Pigliucci, M. (2006). Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecology Letters*, *9*(8), 981–993.

Richardson, D. M., Pyšek, P., & Carlton, J. T. (2011). A compendium of essential concepts and terminology in invasion ecology. *Fifty years of invasion ecology: the legacy of Charles Elton*, 409–420.

Richardson, D. M., Pyšek, P., Rejmánek, M., Barbour, M. G., Panetta, F. D., & West, C. J. (2000). Naturalization and invasion of alien plants: Concepts and definitions. *Diversity and distributions*, *6*(2), 93–107.

Roberts, E. H., & Totterdell, S. (1981). Seed dormancy in rumex species in response to environmental factors. *Plant, Cell and Environment*, *4*(2), 97–106.

Sattin, M., & Sartorato, I. (1997). Role of seedling growth on weed-crop competition. In *Proceedings of the 10th ewrs (european weed research society) symposium, poznań, poland, 22-26 june 1997.*

Schwartz, M. D. (1994). Monitoring global change with phenology: The case of the spring green wave. *International journal of biometeorology*, *38*(1), 18–22.

Shirk, R. Y., Hamrick, J. L., Zhang, C., & Qiang, S. (2014). Patterns of genetic diversity reveal multiple introductions and recurrent founder effects during range expansion in invasive populations of Geranium carolinianum (Geraniaceae). *Heredity*, *112*(5), 497–507.

Steinbauer, G. P., & Grigsby, B. (1957). Interaction of temperature, light, and moistening agent in the germination of weed seeds. *Weeds*, *5*, 175–182.

Tester, M., & Morris, C. (1987). The penetration of light through soil. *Plant, Cell, and Environment*, *10*, 281–286.

Tilman, D., & Lehman, C. (2001). Human-caused environmental change: Impacts on plant diversity and evolution. *Proceedings of the National Academy of Sciences*, *98*(10), 5433–5440.

Toole, V. K. (1963). Light control of seed germination. *Association of Official Seed Analysts*, *53*, 124–143.

Uva, R. H., Neal, J. C., & DiTomaso, J. M. (1997). *Weeds of the northeast*. Cornell University Press.

van Kleunen, M., Weber, E., & Fischer, M. (2010). A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters*, *13*(2), 235–245.

Vitasse, Y., Delzon, S., Bresson, C. C., Michalet, R., & Kremer, A. (2009). Altitudinal differentiation in growth and phenology among populations of temperate-zone tree species growing in a common garden. *Canadian Journal of Forest Research*, *39*(7), 1259–1269.

Weber, E., & Schmid, B. (1998). Latitudinal population differentiation in two species of Solidago (Asteraceae) introduced into Europe. *American Journal of Botany*, *85*(8), 1110–1121.

Weiss-Lehman, C., Hufbauer, R. A., & Melbourne, B. A. (2017). Rapid trait evolution drives increased speed and variance in experimental range expansions. *Nature Communications*, *8*, 1–7.

Williams, J. L., Auge, H., & Maron, J. L. (2008). Different gardens, different results: Native and introduced populations exhibit contrasting phenotypes across common gardens. *Oecologia*, *157*(2), 239–248.

Willis, C. G., Ruhfel, B., Primack, R. B., Miller-Rushing, A. J., & Davis, C. C. (2008). Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(44), 17029–17033.

Wolfe, B. E., Rodgers, V. L., Stinson, K. A., & Pringle, A. (2008). The invasive plant alliaria petiolata (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. *Journal of Ecology*, *96*(4), 777–783.

Wolkovich, E. M., & Cleland, E. E. (2014). Phenological niches and the future of invaded ecosystems with climate change. *AoB plants*, *6*.

Wolkovich, E. M., Davies, T. J., Schaefer, H., Cleland, E. E., Cook, B. I., Travers, S. E., Willis, C. G., & Davis, C. C. (2013). Temperature-dependent shifts in phenology contribute to the success of exotic species with climate change. *American Journal of Botany*, *100*(7), 1407–1421.

Wulff, R. D., Caceres, A., & Schmitt, J. (1994). Seed and seedling responses to maternal and offspring environments in plantago lanceolata. *Functional Ecology*, 763–769.