

SEEDLING SURVIVAL IN A VARIABLE ENVIRONMENT:
CONNECTING PHYSIOLOGY ACROSS LIFE CYCLE STAGES

by

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As members of the Master's Committee, we certify that we have read the thesis prepared by *Ursula Basinger*, titled *Seedling survival in a variable environment: connecting physiology across life cycle stages* and recommend that it be accepted as fulfilling the thesis requirement for the Master's Degree.



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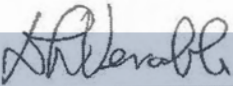


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Final approval and acceptance of this thesis is contingent upon the candidate's submission of the final copies of the thesis to the Graduate College.

I hereby certify that I have read this thesis prepared under my direction and recommend that it be accepted as fulfilling the Master's requirement.



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ABSTRACT

Seedling establishment is a crucial phase when plants are particularly vulnerable to environmental stress. Nonetheless, seedling traits and survival are rarely studied in functional ecology. Using five common species in a well-studied guild of desert annuals, I measured seedling survival and functional traits in response to drought under three temperature regimes in growth chambers that spanned the range of temperatures seedlings normally experience during the germination season. I compared seedling longevity and growth rates to a suite of previously measured plant traits to determine how seedling responses to temperature and drought correspond to physiology at other life cycle stages, as well as to long-term patterns of seedling survival in the field. Generally, seedlings of all species survived longer in cooler temperatures. However, some seedlings had limited early mortality prior to drought conditions, especially in colder temperatures. Temperature-dependent patterns of survival were strongly related to traits expressed at different points in the life cycle, including seed size, germination and, to a lesser extent, growth traits, indicating coordinated functional strategies. Species with larger seeds, faster germination and higher water-use efficiency survived drought longer, especially in the colder temperature. Seedling survival in the growth chamber was correlated with seedling survival rates in the field. Neither relative growth rate nor specific leaf area plasticity under experimental drought conditions connected meaningfully to other functional traits and further research is needed to determine how seedling growth under stress relates to these traits. This study is the first to show that temperature-dependent seedling survival under drought conditions covaries with functional strategies across the lifecycle and predicts natural patterns of establishment in the field.

INTRODUCTION

Understanding community dynamics and patterns of biodiversity requires a mechanistic understanding of not only species' responses to the environment but also how these responses are dependent on life cycle stages (Engelbrecht et al. 2007, Kraft et al. 2015). Research on functional traits holds promise to predict environmental responses (McGill et al. 2006). There is often a focus on one prominent aspect of the life cycle, such as adult growth or reproduction, yet individual fitness and species persistence depends on performance throughout the life cycle (Adler et al. 2014). Response to environmental stress at early stages in particular can have a profound effect on organisms' subsequent life history trajectories (Donohue et al. 2010) and is particularly important to understand but rarely studied.

For a plant, the period of seedling establishment is particularly perilous and survival at this stage depends critically on favorable conditions (Donohue et al. 2010, Burghardt et al. 2015). Thus, plants are under strong selection to evolve germination cues that synchronize seedling emergence with favorable post-germination conditions (Willis et al. 2014). For example, winter annual seeds have a summer after-ripening requirement which helps them avoid germination in summer heat. As ambient temperatures decline in the fall, after-ripening simultaneously widens the range of permissive germination temperatures (Huang et al. 2016). The rate of after-ripening and widening of permissible temperatures presumably evolved as a cue to synchronize germination to suitable conditions for growth, survival, and reproduction (Finch-Savage & Leubner-Metzger 2006). Since suitable conditions are not completely predictable, winter annual plants have also developed species-specific strategies that balance predictive germination and bet hedging both within and across seasons (Gremer et al. 2016). Thus germination begins a contingent developmental trajectory in which the environment at each life stage affects demographic, morphological and physiological traits and selection at subsequent stages (Donohue et al. 2010). Because of the cascading consequences that begin with germination, organisms have evolved complex linkages between germination and other stages. For instance, various genes that regulate flowering time in *Arabidopsis* regulate other ecological traits including dormancy (Chiang et al. 2009, Huo et al. 2016) and dehydration avoidance (McKay et al. 2003). Seedling survival, the critical transition that connects germination ecology and evolution to plant growth, survival and reproduction, is crucial to population persistence.

Survival immediately following germination is modulated by several tradeoffs. One of these is the tradeoff between seed size and number. Larger seeds generally have an advantage in establishment since they are better equipped with resources (Westoby et al. 1996, Metz et al. 2010) but small-seeded plants may be able to make up for lower survival rates by producing more seeds (Moles and Westoby 2004b, Huang et al. 2016). Another tradeoff regulating seedling survival is germination time. Early-emerging seedlings may have greater access to resources (Lortie and Turkington 2002, Tielbörger and Prasse 2009, Donohue et al. 2010, Mercer et al. 2011), escape seed predators and diseases (Weekley et al. 2007, Abe et al. 2008), or outperform later germinants through space and resource preemption (Verdú and Traveset 2005, Mercer et al. 2011, Levine et al. 2011). However, later germinating seedlings can bypass exposure to early season stresses such as drought and herbivory (Petrú et al. 2006, Donohue et al. 2010, Mercer et al. 2011). Risks associated with the timing of germination in the field can vary such that either earlier or later germinating seedlings may be more successful in a given year (Gremer et al. 2016). Two open questions with implications for species coexistence and diversity are whether species differ in their ability to deal with early versus late season seedling risks, and what traits relate to these patterns.

Climate change introduces new challenges by shifting environmental cues for life history transitions in potentially non-adaptive ways. Precipitation patterns are changing worldwide and are key drivers of phenological response to climate change (Peñuelas et al. 2004, Cleland et al. 2006, Franks et al. 2007, Levine et al. 2008). Changes in precipitation can impact community composition by altering germination, seedling establishment, growth, and survival (Lloret et al. 2009, Brown et al. 1997, Weltzin et al. 2003, Miranda et al. 2009). Changes in the timing, rather than the total amount, of rainfall has a greater influence on community composition in some studies (Levine et al. 2008, Kimball et al. 2010, Fay et al. 2011). One response to climate change is increased plant mortality from drought stress, which is well-documented for trees in the western United States (Sala and Hoch 2009, Adams et al. 2009, McDowell et al. 2011). Drought sensitivity can determine species distribution (Engelbrecht et al. 2007) and warmer temperatures drive faster mortality (Adams et al. 2009). Under climate change, increased temperatures and prolonged drought in many regions exacerbate the risk of mortality. Seedlings are especially vulnerable to drought (Baskin and Baskin 2014) but how germination timing interacts with drought and affects community dynamics is largely unknown.

Sonoran Desert winter annual plants have been demographically monitored in southern Arizona for over three decades and community dynamics of this system can now be linked to underlying physiological mechanisms, making them ideal for studying drought-dependent mortality in the context of community ecology (Venable & Kimball 2013). Species differences in germination niche are known to be a primary driver of community dynamics in this system (Baskin et al. 1993, Facelli et al. 2005, Angert et al. 2009, Kimball et al. 2010, Huang et al. 2016). Among-species variation in germination physiology drives differences in germination responses to temperature and water availability and correlates with long-term patterns of germination in the field and growth physiology (Huang et al. 2016). Germination timing relates directly to growth physiology, such that species with higher water-use efficiency (WUE) germinate and reproduce earlier in any given year (Kimball et al. 2011). There is also a growth physiology tradeoff between WUE and relative growth rate (RGR). Species with higher RGR have more plasticity, which allows them to respond to large resource pulses with growth (Angert et al. 2010) but also leads to greater demographic fluctuations (Angert et al. 2007). The more water-use efficient, buffered species are more specialized to low-temperature photosynthetic performance (Gremer et al. 2012). These differences in growth physiology and germination promote species-specific interannual variation in demographic rates among species which, in turn, contribute to species coexistence in this system (Angert et al. 2007, 2009, Huxman et al. 2008). However, as with most systems, much less is known about functional trait expression in seedlings and how it relates to survival and performance very early in the life cycle.

To better understand the seedling establishment niche, I conducted an experiment to compare how five species with diverse germination and growth physiologies respond to immediate post-germination drought stress in different temperature environments that, in nature, may be determined by germination timing. I compared species' survival and growth responses to drought at different temperatures. Further, I investigated relationships between seedling survival or growth and traits expressed at different stages of the life cycle, including seed size, germination physiology, and growth physiology, as well as germination and seedling survival in the field. I predicted that (1) all species would survive longest under colder temperatures because of reduced evapotranspiration rates and that (2) seedling survival variance among temperatures would be explained by known species differences in germination and growth physiology, indicating coordinated adaptive strategies. Assuming that the growth chamber drought conditions would be

a good proxy for unfavorable conditions in the field, I also expected that (3) higher average mortality rates in the field would predict faster mortality in the growth chambers. Lastly, based on the known plasticity of high RGR species in response to rain pulses, I predicted that (4) species with high RGR would be more plastic in growth rates and specific leaf area in response to temperature than species with high WUE.

METHODS

Seedling drought experiment

For this experiment, we focused on five common and abundant species in the Sonoran Desert winter annual community that represent a spectrum of traits, including germination timing and physiology, seed size, and adult physiological and functional traits. These were *Erodium cicutarium* (referred to as ERCI), *Evax multicaulis* (EVMU), *Pectocarya recurvata* (PERE), *Plantago insularis* (PLIN), and *Stylocline microphylla* (STMI) (Table 1a). Seeds for the experiment were collected upon maturity in spring 2013 and after-ripened at the long-term study site over the summer to break physiological dormancy.

Seeds were germinated on agarose gel in sealed petri dishes in a growth chamber set to 18° C in October 2013. Germination, defined as radicle emergence, was checked twice daily and germinants were immediately transplanted to square pots measuring 8 cm wide and 9.5 cm tall and placed in a growth chamber. Pots were filled with three parts Sunshine Mix #3 low-charge soil to two parts 60 grit silica sand, a mix that is similar to desert soils in terms of nutrient availability and soil moisture retention, then watered to saturation. We planted three individuals of the same species in each pot with 25 replicate pots per temperature (75 seedlings/species/temperature, except EVMU, which germinated poorly and had 48-51 seedlings/temperature). Individuals were planted four cm apart, an adequate distance to avoid competition between individuals (Pantastico-Caldas and Venable 2003), especially considering the small sizes reached by the seedlings in this experiment; aboveground measurements rarely reached two cm in height or width.

Three Conviron E7/2 growth chambers (Controlled Environments Ltd, Winnipeg, Manitoba, Canada) were programmed with conditions that represent the full range of temperatures winter annuals may experience during seedling establishment across the germination season. Average temperatures in the three growth chamber were 21 °C, 16 °C, and 11 °C, which are the monthly average temperatures in the field in

October, November, and December-January, respectively. In each chamber, temperatures were gradually raised to 5 °C above average during the day and lowered to 5 °C below average during the night. Bright retrofitted lights provided an irradiance of 1,200 mmol m⁻² s⁻¹ for 12/12-hour light/dark cycles. Pots were given 5 ml water four days after the transplant to aid seedling establishment, and not watered again.

Pots were massed every other day to track moisture loss due to evapotranspiration and then rerandomized within their chamber. Seedlings were tracked daily for viability and scored on a scale of one (fully green and alive) to five (faded to red, dry and dead). When seedlings were determined to be dead (score of five), aboveground mass was removed from the pot, dried and weighed.

Every three days one pot of each species and temperature was randomly chosen for harvest. Aboveground fresh and dry mass were measured for each plant. Total area of cotyledons and leaves was measured using a Licor 3100 Leaf Area Scanner (Licor, Nebraska, USA) for the seedlings in each pot together because the seedlings were too small to measure individually. Seedling relative growth rate was calculated with sequential harvest data as the slope of the natural log of aboveground biomass plotted against age. Specific leaf area at each sequential harvest was calculated as the ratio of total leaf area (cm) to total aboveground dry mass (mg), which was mostly leaves and cotyledons with a very small amount of green photosynthetic stem tissue, both measured as per-pot totals (the sum of three seedlings).

Functional trait and field data

To determine how seedling establishment connects to other life history phases, I compared mortality response and seedling growth patterns to a suite of previously measured germination and growth traits known to be drivers of community dynamics, coded as continuous variables (Tables 1a and 1b). Three laboratory-determined germination traits were used (Liu et al. *in review*, Huang et al. 2016). The first, germination rate to 16% (GR(16)), is the inverse of hours to 16% germination on filter paper moistened with pure water (0 MPa) at 10 °C. The value of 16% was used because it is close to the first standard deviation from the mean and a strong determinant of overall germination rate. The next germination trait was maximum rate of after-ripening, (AR(16)). Seeds of winter annuals must after-ripen in hot, dry summer conditions before they can germinate in the fall and winter, and these after-ripening requirements vary among species (Huang et al. 2016). AR(16) captures species' capacity for dormancy loss

during summer after-ripening as the maximum jump in GR(16) from one month of after-ripening to the next. As a final germination trait, I used log transformed hydrothermal time to germination ($\ln(\Theta_{HT})$). Θ_{HT} is calculated as the product of degrees of ambient temperature above base temperature, ambient water potential above base water potential (MPa), and time to 50% germination (Liu et al. *in review*). Seeds with higher values of $\ln(\Theta_{HT})$ germinate more slowly. For metrics of growth traits, I used a principal component (PC) score based on five physiological traits underlying a tradeoff between relative growth rate (RGR) and water-use efficiency (WUE) calculated by Angert et al. (2009; Table 1b). High values of this growth physiology PC represent species with low RGR and high WUE, and low values represent species with high RGR and low WUE. To describe field germination behavior, I calculated a field germination PC that indexes germination propensity (Table 1b). This PC score summarizes 20-year mean of field germination fractions, 25-year average germination dates (see Huang et al. 2016), and 32-year average proportion early germination within years (see Gremer et al. 2016). For this PC index, faster- and earlier-germinating and high-germination-fraction species have a higher score. Last, I compared seedling performance to species' average seed masses, a metric widely believed to confer an advantage in establishment (Grime et al. 1981, Cornelissen 1999, Westoby et al. 2002).

Statistical analyses

Mortality in the growth chamber

To test predictions 1 and 2 and determine how seedling survival at different temperatures relates to species differences in physiology and ecological niche, I analyzed the impact of temperature on days alive using a two-way ANOVA (Proc Mixed, SAS 9.4, SAS Institute Inc., Cary, NC). I modeled covariance of plants in the same pots with compound symmetric error covariance. In analyzing the data, I noticed that, while most plants died when soil moisture was greatly depleted, some plants died early with considerable soil moisture still present. I analyzed the data with and without early dying plants to determine whether some species and temperature environments tended to produce more early deaths than others.

To determine what correlations between temperature-driven drought response and functional traits, I repeated the two-way ANOVAs as ANCOVAs with species coded by their species-mean functional trait values (continuous variables) both with and without early dying seedlings (Proc Mixed, SAS 9.4). For

functional trait values I used GR(16), AR(16), $\ln(\Theta_{HT})$, growth physiology PC score, field germination PC score, and seed size.

Soil moisture availability

To test whether death rate was a function of water availability, the proportion of water lost was calculated for each pot containing three plants at the time of each plant's death as [(initial water mass - water mass at death) / (initial water mass)]. The ratios were then normalized so that the values across the entire experiment ranged from zero, when the plants with the most water available died, to one, when the plants with the least amount of water available died. Thus, the range for each species or temperature group is less than one and demonstrates relative soil dryness among all pots in the experiment. To see if species or temperature regimes had different means or variances for normalized soil moisture loss at death I used Welch's test for one-way ANOVA and Levene's Test for homogeneity of variances (Proc GLM, SAS 9.4).

Mortality in the field

To determine if seedling mortality patterns in the growth chamber reflect those observed in the field (prediction 3), I calculated early mortality (i.e., seedling mortality) for cohorts at different periods in the growing season using long-term demographic data from 1982-2016. For each year, plants of each species were sorted into germination cohorts (germination in response to a single rain pulse). Seedling mortality was identified as mortality between the germination census and the following census, which occurred 20 to 60 days later, depending on the cohort and year, and calculated as a proportion of cohort germination. Then cohorts were grouped into three periods: September and October (early/hot), November (mid/medium), and December and January (late/cold), with period mortality weighted by cohort abundance if there was more than one cohort in a given period. To see if species seedling mortality was distributed differently across germination periods, arcsine square root transformed mortality was analyzed treating species, period, and their interaction as fixed effects and year as a random effect (Proc Mixed in SAS 9.4). To determine if mortality patterns in the field correspond to mortality patterns in the growth chamber, I conducted a second analysis substituting days alive in the growth chamber for species. I did this using days alive averaged across growth chamber temperatures including seedlings that died before the onset of

drought and then again using days alive in the cold growth chamber only, where patterns were strongest. I also repeated the field mortality analysis with species replaced with trait values as done for the growth chamber analysis, which allowed me to determine if mortality patterns in the field and growth chamber are associated with the same physiological syndromes.

Seedling growth rate

To test for variation in seedling RGR among species and temperatures (prediction 4), I regressed the natural log of aboveground biomass against age, including temperature and species as nominal fixed-effects (Proc Mixed in SAS 9.4). Growth rate decline over time was captured with a quadratic term for age. I modeled covariance of plants in the same pots with compound symmetric error covariance. Starting with a full model containing all interactions of the fixed effects I progressively removed nonsignificant higher order interactions until all remaining terms were significant using Type III sums of squares. Next, I repeated the analysis with species coded as continuous functional traits, GR(16), AR(16), $\ln(\theta_{HT})$, growth physiology PC score, field germination PC score, and seed size.

Specific leaf area

To see how SLA changed during the dry down and how this change varied with temperature and species (prediction 4), I regressed the natural log of leaf area ($\ln(LA)$) against the natural log of dry mass ($\ln(DM)$) including temperature and species as nominal covariates with outliers removed (Proc Mixed in SAS 9.4). I subtracted the mean value of $\ln(DM)$ from pot $\ln(DM)$ for the analysis. Using this deviation from mean as the x-variable has no effect on the slope but moves the intercept to mean $\ln(DM)$, where it is more intuitively interpretable than at zero $\ln(DM)$. Specific leaf area is the slope of leaf area plotted against dry mass (on arithmetic axes). On log-log axes, a slope of one would indicate no change in SLA across development, while slopes differing from one would indicate allometric differences in SLA over the course of the dry down. Differences in intercept between species or temperatures indicate higher or lower SLA evaluated at the geometric mean plant dry weight. The three-way interaction of $\ln(DM)$ with temperature and species indicates how species differ in their temperature plasticity for specific leaf area response to dry down. To determine how individual species responded to temperature, I also performed a regression for

each species separately on $\ln(LA)$ as a function of $\ln(DM)$ including temperature as a nominal variable. Since the leaf area and mass for seedlings in the same pot were combined and pots were assumed to be independent, I did not need to correct for correlated errors as in the growth rate analysis.

RESULTS

Mortality in the growth chamber

Seedlings survived longest in the colder temperature treatment and least in the warmest temperature, both by about 10 days relative to medium temperature (temperature main effect with early dying seedlings removed, $P < 0.001$; Table 2a). Some species survived longer overall than others (species main effect, $P < 0.001$). Mortality responses to temperature differed among species (species by temperature interaction, $P = 0.0122$).

When I repeated this analysis with early dying seedlings included I found the same trends. Most early-dying seedlings were STMI and EVMU in the colder temperatures. A few EVMU and STMI also died early in the two warmer temperature regimes and a few ERCI and PLIN died exclusively in the colder regime, but no PERE died prematurely (Table 2b). Species survived longest in the colder temperature regime (temperature main effect, $P < 0.001$), and there was an overall trend for the same species to outlive others regardless of temperature (species main effect, $P < 0.001$). The differences in species responses to temperature were even greater with the inclusion of these early dying seedlings (species by temperature interaction, $P = 0.0002$), primarily because this lowered the average time alive for EVMU and STMI in the colder temperature regime.

When I coded species using species mean functional trait values as continuous variables, the main effects generally followed a similar pattern both with and without early-dying seedlings (Fig. 1). Effects of functional traits were stronger with the inclusion of the early-dying seedlings (Table 2a). As before, growth chamber temperature significantly impacted days alive in the context of all variables, with or without early dying seedlings (Fig. 1, Table 2a, $p < 0.0001$ for all variables). Species germination physiology variables were significantly correlated with days alive (Table 2a). Species with higher GR(16) (with and without early-dying seedlings included; Fig. 1a), higher AR(16) (only with early-dying seedlings included; Fig. 1b),

and lower $\ln(\theta_{HT})$ (only with early-dying seedlings included; Fig. 1c) lived longer. These patterns indicate that species with germination physiology parameters that promote faster germination survived longer. Higher growth phase physiology PC also positively predicted days alive under drought (Fig. 1d), indicating that species with higher WUE and lower RGR staved off drought effects longer. Higher field germination PC (higher germination fraction, earlier germination date; Fig. 1e) positively predicted days alive in the growth chamber as well. Lastly, higher seed mass had a weak positive effect on days alive but only with the early-dying seedlings included (Fig. 1f). To summarize, all functional traits significantly predicted days alive when early-dying seedlings were included and three of the six functional traits significantly predicted days alive when early dying seedlings were removed.

Species-specific seedling mortality response to temperature also related to functional traits. The interaction between species traits and temperature was strengthened by including all individuals in the analysis (Fig. 1, Table 2a). With early dying seedlings included, all traits showed a highly significant interaction with temperature ($P < 0.001$), except for seed mass, which was weakly significant ($P < 0.05$). When the early dying seedlings were not included, AR(16), field germination PC and seed mass weakly interacted with temperature ($P < 0.05$), and the other three variables did not. The interaction effects were largely due to the colder treatment, which had more differentiation in survival among species. The trait values associated with longer life in the colder temperature were the same trait values associated with longer life as main effects. For example, species with higher GR(16) lived longer in general (main effect) and had the highest longevity sensitivity in the colder treatment relative to the warmer treatments (species by temperature interaction; Fig. 1).

Soil moisture availability

For the analysis of soil moisture at death from drought, I removed the plants that died with ample soil moisture still available. Individuals died from drought at very similar soil moisture availability regardless of species or temperature ($F_{14,185} = 0.80$, $P = 0.6652$; Fig. 2). Likewise, the variance in mean soil moisture at time of death did not depend on species or temperature ($F_{14,185} = 1.37$, $P = 0.1725$). Thus, while total days alive did differ among species and temperatures, most plants died at the same soil moisture level, which was simply reached at different rates.

Mortality in the field

There was a significant difference in mortality between seedlings germinating at different seasonal time periods in the field, with highest 33-year average early mortality for the earliest germinants (September/October, 50%, Fig. 3) and lower early-life-cycle mortality for both mid (November) and late (December/January) germinants (20%, timing main effect $P < 0.0001$, Table 3). Survival also varied among species (species main effect $P = 0.0227$). Species differences in early life cycle mortality in the field were most pronounced for seedlings that germinated early in the season, when temperatures are warmer, and were also evident for late-season germinants but no differences were observed for mid-season germinants (species by timing interaction $P = 0.0064$; Fig. 3).

Field mortality correlated strongly with the patterns of mortality in the growth chamber (average days alive main effect, $P = 0.0064$), and even more strongly with days alive in the cold temperature treatment (average 11 °C days alive main effect, $P = 0.0011$; Fig. 3). The small-seeded, slow-germinating, fast-growing species that have low germination fractions and tend to germinate later in the season suffered the greatest seedling mortality within early- and late-season germination groups, as seen with the significant main effect and interaction of season with every physiology variable and field germination PC (Table 3).

Seedling growth rate

Seedlings achieved larger early sizes in the warmer temperatures as reflected in significant differences in the growth curve intercept (temperature main effect $P < .0001$; Table 4a; Fig 4a). Yet over the course of the dry-down, seedlings grew fastest in cold and slowest in hot temperatures (age by temperature interaction, $P = 0.0001$). Regardless of temperature, growth rate differed among species (age by species interaction, $P = 0.0061$), and was higher for PERE and EVMU and lower for STMI, ERCI and PLIN. Also, growth rate decreased over time in general as water became scarce and individuals became progressively more stressed (quadratic age term, $P < 0.0001$). However, the growth rate difference among species was similar at different temperatures (age by temperature by species interaction, $P = 0.784$) and the

rate of growth rate decline did not vary among species or temperatures (interactions involving age² were not significant).

Variation in seedling growth rate was only correlated with germination in the field. When species were coded as functional traits or field germination PC, overall patterns were maintained, but growth rate did not correlate with the species mean values for any trait except field germination PC (age by field germination PC interaction, $F(1,140) = 3.93$, $P = 0.049$; Table 4a). The fastest and earliest germinators in the field generally exhibited a faster growth rate under experimental water stress conditions regardless of experimental temperature. All traits correlated with initial seedling size (species main effect, Table 4a). Species with greater seed mass had greater initial seedling sizes (seed mass main effect $F(1,141) = 350.63$, $P < 0.001$) and other variables' effect on initial seedling size could be due to their correlation with seed size.

Specific leaf area

The trajectory of SLA through development and dry down differed among species (dry mass by species interaction, $P < .001$; Table 4b) and temperature and species (dry mass by temperature by species interaction, $P = 0.0077$; Fig. 3b). This indicates that species differed in phenotypic plasticity for specific leaf area over time in ways that varied across temperatures (Table 4b). Slopes of $\ln(\text{LA})$ over $\ln(\text{DM})$ were usually, but not always less than or equal to one, indicating either no change in SLA during dry down (slope = 1) or decline in SLA with dry down (slope < 1). The slope for STMI was greater than one in the cold temperatures, indicating that SLA actually increased over time at this temperature regime while it remained unchanged over dry down in medium temperature and declined over the experiment in the hot treatment. SLA did not change much over time for PERE in the cold and medium temperatures but declined in the hot temperatures. SLA for ERCI, PLIN and EVMU was unchanged over dry down in the hot temperatures but declined in medium and cold temperatures for ERCI and PLIN and just declined in cold for EVMU. PLIN, which grew little, did not vary in SLA across temperatures. Effects other than this three-way interaction were nonsignificant, except for dry mass, which intrinsically covaries with leaf area through development, and the interaction between dry mass and species (i.e., the species differences in SLA). Species differences were not explained well by functional trait differences or field germination behavior.

DISCUSSION

Even small physiological differences in seedling growth and survival can lead to large differences in community structure in unpredictable and changing environments. For seedlings in arid environments, the ability to endure one more day with low soil moisture may be all it takes to ensure survival. Despite the importance of the seedling stage for individual performance and population dynamics, how functional traits at this stage relate to patterns of establishment and response to environmental stress is largely unknown. I tackled this gap in knowledge by experimentally determining species' unique seedling responses to water and temperature stress under controlled conditions. Further, I explored whether there was concordance among functional traits expressed at different stages of the life cycle, from seeds, to seedlings, to adult plants. I found that, although neither seedling growth rate nor SLA differences among species showed straightforward patterns, seedling longevity under dry down in the growth chamber and seedling mortality in the field adaptively correspond to known species trait differences in seeds, germination, and adult growth. Species that withstood drought longer in the growth chamber generally have greater survival rates in the field, larger seeds, faster germination rates, and higher WUE. Thus, this study provides evidence for adaptive linkages between seedling establishment and other life history traits.

Predictions 1 and 2: All species should survive longest under the cold temperature and seedling survival variance among temperatures should correlate with known species differences in germination and growth physiology.

Overall, seedlings lived longer in cooler treatments, as predicted. Evaporation and transpiration are strongly temperature-dependent in the field (McDowell et al. 2008, Allen et al. 2010, Wang et al. 2018) and in growth chambers (Adams et al. 2009, McDowell et al. 2016), so moisture loss is expected to be lower at cooler temperatures. While I expected this reduction in evapotranspiration to benefit all species, I also expected that certain species would benefit more from cooler temperatures, based on known physiological traits and germination timing observed in the field. I expected higher WUE species to perform better under colder conditions because they maximize photosynthesis at lower temperatures

(Gremer et al. 2012, Huxman et al. 2013). Knowing that high WUE species also have higher germination fractions and larger seeds, we also expected species with these traits to perform better as seedlings under colder conditions (Huang et al. 2016). As anticipated, high WUE, fast germinating, and large-seeded species survived longer in cooler temperatures.

Species differences in seedling survival are interesting in the context of climate change in the Sonoran Desert. Over the last 35 years germination rains have tended to occur later in the season when conditions are cooler. As a result, cold-adapted, high water-use-efficient plants have become more common because of their ability to both germinate quickly and photosynthesize in cold temperatures (Kimball et al. 2010, Ge et al. 2018). This experiment provides evidence that the cold temperature advantage extends to the seedling establishment stage.

Species differences were more accentuated in the cold and the effect of temperature on species was correlated with several functional traits. When early dying seedlings were excluded from the analysis, larger seed size and faster after-ripening replaced growth physiology PC and germination rate as the most important factors responsible for greater longevity differences in cold. Faster after-ripening (AR(16)) is correlated with faster germination (GR(16)) and growth physiology PC is correlated with seed size, so this switch in the strongest predicting traits does not represent a dramatic shift in explanatory factors with and without the early-dying seedlings included. Other studies have shown seedling survival in response to abiotic environmental changes for single species (Huxman et al. 1998, Duan et al. 2018). I show that this response is variable among species in a community.

It was surprising that some species had considerable mortality even when soil moisture was still high, especially in cold temperatures. Interestingly, species by temperature interactions were stronger overall when these early dying seedlings were included. In the growth chamber, early mortality for some species may be due to either differential transplant stress of new germinants or inherent differences among species in the ability to transition from dormant seed to physiologically self-sustaining seedling. The seedlings that died early before soil moisture reached stressful levels were mostly small-seeded species that had difficulty establishing in the cold even before the onset of drought. This suggests an inherent disadvantage for small-seeded species in the transition from seed to seedling that depends on temperature in addition to water availability. The small-seeded species also have higher germination temperature optima

and do not germinate under cold temperatures as readily, and the stress they experienced early in the experiment was most likely due to cold temperatures rather than drought (Huang et al. 2016). Small-seeded plants are generally found to have lower seedling establishment (Grime and Jeffrey 1965, Westoby et al. 1996, Lloret et al. 1999, Walters and Reich 2000, Metz et al. 2010, Huang et al. 2016) but often make up for this loss by producing more seeds (Leishman 2001, Moles and Westoby 2004a, Huang et al. 2016). The early mortality of small-seeded plants in my experiment supports this seed-size and seedling establishment pattern.

Some species survived drought longer as seedlings, regardless of temperature. These same species have a high germination fraction in the field, large seeds and high WUE. Likewise, species with lower germination fractions, small seeds, and high RGR that died sooner as seedlings in this experiment. My findings suggest coordination of the establishment niche with the germination and growth niches. Slower and delayed germination is likely an adaptation to the greater risk inherent in not surviving as well.

My results correspond with other studies that have shown general trait syndromes that link to the seedling stage. For instance, smaller seeded species can have higher RGR and more surface area as seedlings (Paz et al. 2005, Fenner and Thompson 2005). It has been suggested that these traits contribute to increased seedling mortality rates (Moles and Leishman 2008), which is what I observed in this study. One study demonstrated a multidimensional relationship between seedling, germination, and seed traits in 47 species of grasses in the growth chamber, although these traits did not predict seedling behavior in the field (Larson et al. 2016). Seedling functional traits can also correlate with the same traits at the adult stage (Cornelissen et al. 2003). However, one study found discrepancies between seedling and adult survival among species under drought (Lloret et al. 2009). Given the evidence for seedling establishment as an important stage that is subject to great environmental filtering (Grubb 1977, Fernández-Pascual et al. 2017) and influences subsequent traits (Donohue 2014), it is surprising that more is not known about the effect of drought on seedling survival in relation to other traits across the life cycle. My study begins to fill this gap in understanding by connecting seedling mortality under environmental stress to seed, germination, and growth physiology traits for multiple species in a community.

Soil water availability at the time of death was invariant across species and temperatures. Yet there were still species and temperature differences in seedling longevity because water evaporated from the soil

more quickly at warm temperatures and transpiration varied with temperature at species-specific rates. This temperature-dependent usage of soil moisture contrasts with results for pinyon seedlings that used water at the same rate regardless of temperature (Adams et al. 2009), but is similar to results for eucalypts where, under different temperatures and CO₂ levels, plants reached soil moisture limitation at different dates (Duan et al. 2018). My results suggest that soil moisture limits for survival of these tiny annual plants are similar, but that species are able to use available water at different rates, possibly due to differences in root expansion rates or stomatal control.

Prediction 3: Growth chamber time to mortality should accurately predict seedling mortality patterns observed in the field.

Seedling mortality in the field also appeared to adaptively correspond to germination and plant-growth-phase species differences. Mortality differences among species were most pronounced for seedling cohorts that germinated early in the season when temperatures are typically warmer, and, to a slightly lesser degree, for seedlings that germinated late in the season when conditions are cooler. Midseason germinating cohorts had low mortality regardless of species. The species with high early- and late-season mortality in the field were small-seeded, slow-germinating, fast-growing species that have low germination fractions, tend to germinate later in the season in any given year, and had the fastest mortality rates in the cold growth chamber treatment. Few studies have linked seedling survival in the field and under experimental drought conditions. One exception to this is a grassland study that measured seedling survival under greenhouse drought regimes to predict establishment in the field (Frasier 1989). To my knowledge, my study is the first to link species variation in drought mortality in the lab to long-term seedling mortality trends in the field.

Prediction 4: Species should exhibit temperature-driven differences in plasticity in growth rate and specific leaf area.

My results generally support the assertion that germination and seedling establishment are adaptively coordinated (Grubb 1977, Leck et al. 2008, Donohue et al. 2010). Species that germinated earlier and faster in the field (high field germination PC) showed a tendency to grow faster under drought as seedlings. However, the other traits I measured did not predict variation in seedling growth rates,

suggesting that a mechanistic explanation for the connection between germination in the field and seedling growth under drought may be in factors we did not measure. Initial seedling size correlated well with seed size, as has been widely observed in other studies (Grime et al. 1981, Cornelissen 1999, Westoby et al. 2002). Seedling growth rate, however, did not relate to seed size, going against the general assumption that smaller seeded plants have greater RGR (Turnbull et al. 2012). Seedling growth rate also did not seem to correlate with days alive, indicating that growth strategy and survival are not aligned in a way that can be easily understood.

Functional traits are expected to drive responses to environmental conditions (McGill et al. 2006, Suding et al. 2008). For example, plants regulate photosynthesis in response to light and water availability by adjusting SLA (Dwyer et al. 2014, Gratani 2014). SLA can also be temperature-dependent (Rosbakh et al. 2015, Sandel et al. 2016). In my study, seedling SLA responses to temperature varied across species, indicating species differences in temperature plasticity. These species differences in plasticity did not relate to functional traits nor days alive, suggesting that species' SLA responses to drought stress at different temperatures may have different fitness consequences for different species. For example, SLA for STMI increased through dry down in cold temperatures and decreased in warm temperatures, yet STMI did not live long in the cold temperature and has one of the highest temperature optima for photosynthesis in laboratory experiments (Gremer et al. 2012). Perhaps the decline in SLA in hot temperatures facilitated its strong photosynthetic performance. In contrast, EVMU, which also did not live long in cold temperatures in my experiment and had the highest temperature optimum for photosynthesis of all species in Gremer et al. (2012), maintained constant SLA through dry down in the hot temperature but declined in SLA in the cold temperature. These varied responses merit further investigation.

Few studies have measured seedling growth strategies in response to environmental variation. Still, there is evidence that seedling growth responses to temperature are species-specific (Arnold 1974). Rarer yet are studies that measure seedling growth under drought, and those that have focused on root growth allocation in response to drought (Padilla et al. 2007, Larson and Funk 2016). Since plastic responses are weaker in unfavorable conditions (Angert et al. 2010) and seedlings may exhibit less plasticity than adults (Leck et al. 2013), comparing growth traits of drought-stressed seedlings is

challenging and it is not surprising that I did not find clear growth patterns in this short but crucial period in annual plant development.

CONCLUSION

In this study I bridged known seed, germination and growth traits by quantifying their relation to seedling survival under drought conditions. My study is the first to identify multispecies temperature-driven plasticity in seedling mortality response, and to show how these responses correlate with both seedling survival in the field and key seed, germination and growth traits. My findings provide insight into mechanisms driving patterns of population and community dynamics in this winter annual plant community over the last three decades, including responses to contemporary climate change. The seedling stage, albeit brief, is impactful.

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TABLES

Table 1a. Species in the experiment.

Species name used	Code	Family	Current APG IV name	Status
<i>Erodium cicutarium</i>	ERCI	Geraniaceae	<i>Erodium cicutarium</i>	invasive
<i>Evax multicaulis</i>	EVMU	Asteraceae	<i>Evax multicaulis</i>	native
<i>Pectocarya recurvata</i>	PERE	Boraginaceae	<i>Pectocarya recurvata</i>	native
<i>Plantago insularis</i>	PLIN	Plantaginaceae	<i>Plantago ovata</i>	native
<i>Stylocline micropoides</i>	STMI	Asteraceae	<i>Stylocline micropoides</i>	native

Species four-letter abbreviations (Code) listed here.

Table 1b. Species' mean trait values used in ANOVA comparisons.

	ERCI	EVMU	PERE	PLIN	STMI
Germination rate, GR(16)	0.02	0.008	0.03	0.02	0.01
After-ripening rate, AR(16)	0.02	0.00	0.01	0.01	0.01
ln(Hydrothermal time to germination)	5.56	7.67	5.86	6.39	6.37
Growth physiology (PC score)	0.97	-3.16	2.38	1.09	-1.24
Field germination (PC score)	2.17	-3.60	0.01	1.08	-0.66
Seed mass (mg)	2.00	0.03	0.95	0.95	0.13

Table 1c. Traits used in this paper.

Trait group	Variable
Germination physiology	Germination rate, GR(16): Inverse of germination time in hours to 16 percent germination in pure water (0 MPa) at 10 °C After-ripening rate, AR(16): The maximum jump in GR(16) from one month of after-ripening to the next (see Methods for details) Hydrothermal time to germination, logged, $\ln(\theta_{HT})$
Growth physiology PC	- Specific leaf area (leaf area/dry mass) + Leaf mass ratio (leaf dry mass/plant dry mass) - Relative growth rate plasticity after larger rainfall events (change in LAR, see Angert et al. 2007) + Ratio of maximum electron transport to maximum carboxylation velocity (J_{max}/V_{Cmax}), see Huxman et al 2008 + Leaf nitrogen content
Field germination PC	+ Germination fraction: proportion of germination averaged over 25 years + Proportion early germination: proportion of the total year's germination occurring in the first cohort, averaged over 25 years - Mean germination date, averaged over 25 years
Seed size	Seed mass (mg)

Signs of + or - indicates whether the variable increases or decreases with increasing PC score. Higher values GR(16) and AR(16) result in faster germination and higher hydrothermal time leads to slower germination. Higher values of growth physiology PC means higher WUE and lower RGR. Higher field germination PC means greater, earlier germination and lower germination fraction.

Table 2a. Summary of analysis of variance tests of days alive in growth chambers, without and with seedlings that died early with soil moisture available. Parameters given are F-ratios for fixed effects.

	Without early mortality	With early mortality
Species	F(4, 185) = 18.31 ****	F(4, 187) = 19.79 ****
Temperature	F(2, 185) = 2279.39 ****	F(2, 187) = 613.33 ****
Interaction	F(8, 185) = 2.54 *	F(8, 187) = 4.10 ***
GR(16)	F(1, 194) = 5.98 *	F(1, 196) = 34.48 ****
Temperature	F(2, 194) = 231.09 ****	F(2, 196) = 47.07 ****
Interaction	F(2, 194) = 1.53	F(2, 196) = 13.29 ****
AR(16)	F(1, 194) = 2.07	F(1, 196) = 14.58 ***
Temperature	F(2, 194) = 250.36 ****	F(2, 196) = 52.95 ****
Interaction	F(2, 194) = 3.40 *	F(2, 196) = 7.24 ***
ln(Θ_{HT})	F(1, 194) = 1.53	F(1, 196) = 22.62 ****
Temperature	F(2, 194) = 27.52 ****	F(2, 196) = 27.79 ****
Interaction	F(2, 194) = 1.13	F(2, 196) = 10.11 ****
Growth physiology PC	F(1, 194) = 7.98 **	F(1, 196) = 37.46 ****
Temperature	F(2, 194) = 1684.02 ****	F(2, 196) = 529.27 ****
Interaction	F(2, 194) = 1.86	F(2, 196) = 13.52 ****
Field germination PC	F(1, 194) = 11.59 ***	F(1, 196) = 32.06 ****
Temperature	F(2, 194) = 1793.46 ****	F(2, 196) = 525.00 ****
Interaction	F(2, 194) = 3.25 *	F(2, 196) = 9.09 ***
Seed Mass	F(1, 194) = 0.03	F(1, 196) = 4.83 *
Temperature	F(2, 194) = 584.52 ****	F(2, 196) = 135.70 ****
Interaction	F(2, 194) = 3.89 *	F(2, 196) = 4.62 *

**** $p < .0001$, *** $p < .001$, ** $p < .01$, * $p < .05$

Table 2b. Early deaths as a percentage of total seedlings in the growth chamber experiment.

	ERCI	EVMU	PERE	PLIN	STMI
Cold	10.53	29.03	0.00	6.67	13.16
Medium	0.00	3.85	0.00	0.00	5.13
Hot	0.00	13.33	0.00	0.00	0.00

Table 3. Summary of analysis of variance tests of seedling mortality in the field. Parameters given are F-ratios for fixed effects. Average days alive is the average across growth chamber temperatures with early mortality included, and cold average days alive is the same calculation for the cold growth chamber only.

	F-statistic
Species	F(2, 158) = 2.93 *
Temperature	F(4, 158) = 21.92 ****
Interaction	F(8, 158) = 2.15 *
Average days alive	F(1, 167) = 7.63 **
Germination timing	F(2, 167) = 6.19 **
Interaction	F(2, 167) = 5.58 **
Cold average days alive	F(1, 167) = 11.03 **
Germination timing	F(2, 167) = 10.38 ****
Interaction	F(2, 167) = 7.62 ***
GR(16)	F(1, 167) = 7.30 **
Temperature	F(2, 167) = 15.79 ****
Interaction	F(2, 167) = 5.36 **
AR(16)	F(1, 167) = 6.60 **
Temperature	F(2, 167) = 15.89 ****
Interaction	F(2, 167) = 5.06 **
ln(Θ_{HT})	F(1, 167) = 6.70 *
Temperature	F(2, 167) = 3.31 *
Interaction	F(2, 167) = 5.13 **
Growth physiology PC	F(1, 167) = 8.21 **
Temperature	F(2, 167) = 22.92 ****
Interaction	F(2, 167) = 5.95 **
Field germination PC	F(1, 167) = 9.59 **
Temperature	F(2, 167) = 21.87 ****
Interaction	F(2, 167) = 6.91 **
Seed mass	F(1, 167) = 4.10 *
Temperature	F(2, 167) = 16.67 ****
Interaction	F(2, 167) = 3.37 *

**** $p < .0001$, *** $p < .001$, ** $p < .01$, * $p < .05$

Table 4a. Summary of analysis of variance test of relative growth rate (RGR) for species and continuous trait values. Parameters given are F-ratios for fixed effects.

	Species	GR(16)	AR(16)
Age	F(1, 134) = 48.38 ****	F(1, 141) = 13.58 ***	F(1, 141) = 34.90 ****
Age x Age	F(1, 134) = 21.75 ****	F(1, 141) = 5.67 *	F(1, 141) = 16.26 ****
Temperature	F(2, 134) = 11.21 ****	F(2, 141) = 3.48 *	F(2, 141) = 7.85 ***
Species	F(4, 134) = 53.63 ****	F(1, 141) = 214.09 ****	F(1, 141) = 616.51 ****
Age x Temperature	F(2, 134) = 14.97 ****	F(2, 141) = 5.02 **	F(2, 141) = 10.07 ****
Age x Species	F(4, 134) = 3.77 ***	NS	NS
	ln(HTT)	Growth PC	Field PC
Age	F(1, 141) = 18.38 ****	F(1, 141) = 15.73 ****	F(1, 140) = 34.3 ****
Age x Age	F(1, 141) = 8.14 **	F(1, 141) = 6.61 *	F(1, 140) = 15.45 ****
Temperature	F(2, 141) = 4.38 *	F(2, 141) = 4.02	F(2, 140) = 8.11 ***
Species	F(1, 141) = 305.14 ****	F(1, 141) = 266.89 ****	F(1, 140) = 156.8 ****
Age x Temperature	F(2, 141) = 6.01 **	F(2, 141) = 5.78	F(2, 140) = 10.96 ****
Age x Species	NS	NS	F(1, 140) = 3.93 *
	Seed mass		
Age	F(1, 141) = 23.19 ****		
Age x Age	F(1, 141) = 10.91 **		
Temperature	F(2, 141) = 5.17 **		
Species	F(1, 141) = 350.63 ****		
Age x Temperature	F(2, 141) = 6.52 ***		
Age x Species	NS		

**** $p < .0001$, *** $p < .001$, ** $p < .01$, * $p < .05$

Best fit models are presented and removed interactions have values of non-significant (NS).

Table 4b. Summary of analysis of variance test of specific leaf area (SLA). Parameters given are F-ratios for fixed effects.

	F-ratios
Dry mass deviation	F1, 98 = 151.14 ****
Species	F4, 98 = 1.12
Temperature	F2, 98 = 0.85
Dry mass deviation x Species	F4, 98 = 3.63**
Dry mass deviation x Temperature	F2, 98 = 0.09
Species x Temperature	F8, 98 = 1.68
Dry mass deviation x Species x Temperature	F8, 98 = 3.55**

**** $p < .0001$, *** $p < .001$, ** $p < .01$, * $p < .05$

Notes: Results for ANOVAs for SLA with species coded as variables were only significant for ln(DMdev) and are not reported. Dry mass is recoded as dry mass deviation from the mean so that the intercept is evaluated at average plant size.

FIGURES

Figure captions

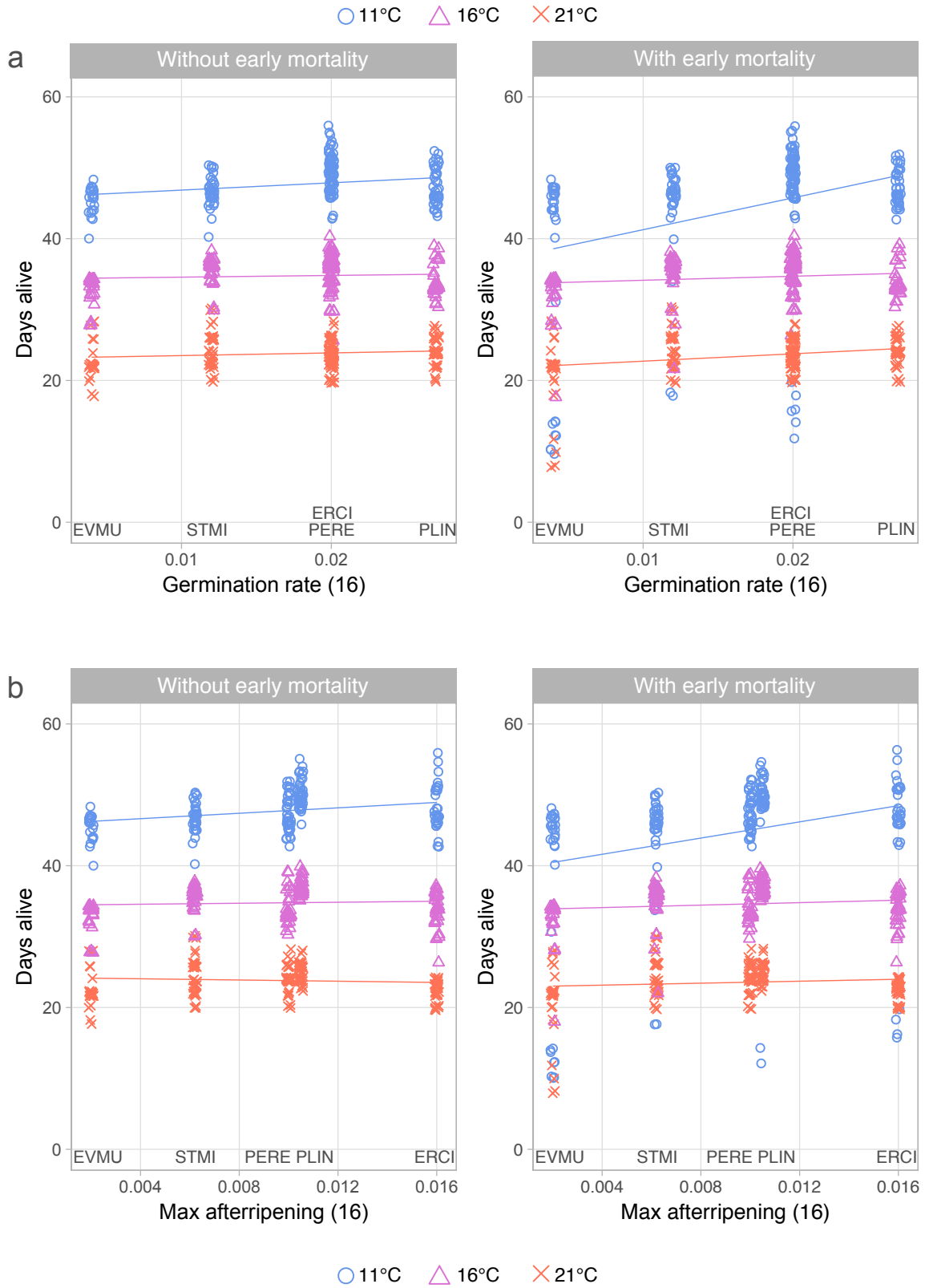
Figure 1. Linear regressions of days alive as a function of various species traits under three growth chamber temperatures. Circles, triangles, and x's represent individual plants in the cold (11 °C average temperature), medium (16 °C average temperature), and hot growth chambers (21 °C average temperature), respectively. Solid, dashed and dotted lines denote linear regressions within the cold, medium, and hot growth chambers, respectively. Individuals that died early are removed from the left graphs and included in the right graphs. Species names are abbreviated with four-letter codes. Traits shown are GR(16), the inverse of hours to 16 percent germination on filter paper moistened with pure water, with germination rate increasing from left to right (a); AR(16), the maximum jump in GR(16) from one month of after-ripening to the next (b); $\ln(\Theta HT)$, the natural log of hydrothermal time to germination (c); growth physiology PC score, with RGR decreasing and WUE increasing from left to right (d); field germination PC score, with higher values signifying faster earlier germination (e); and seed mass (f).

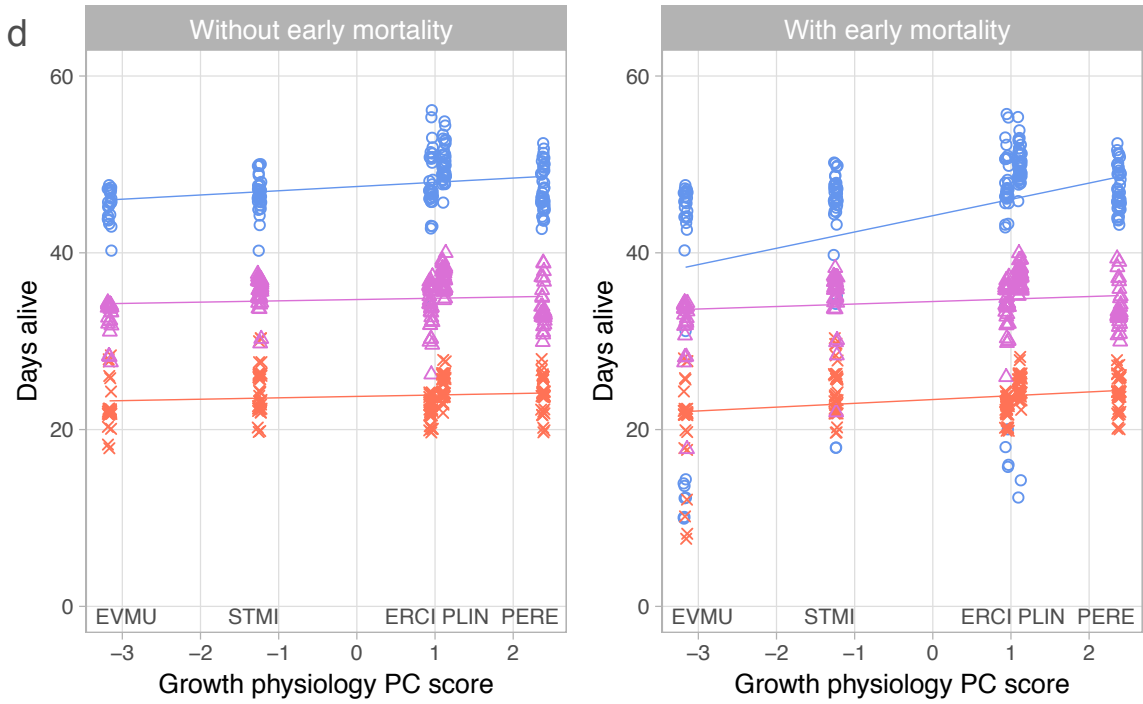
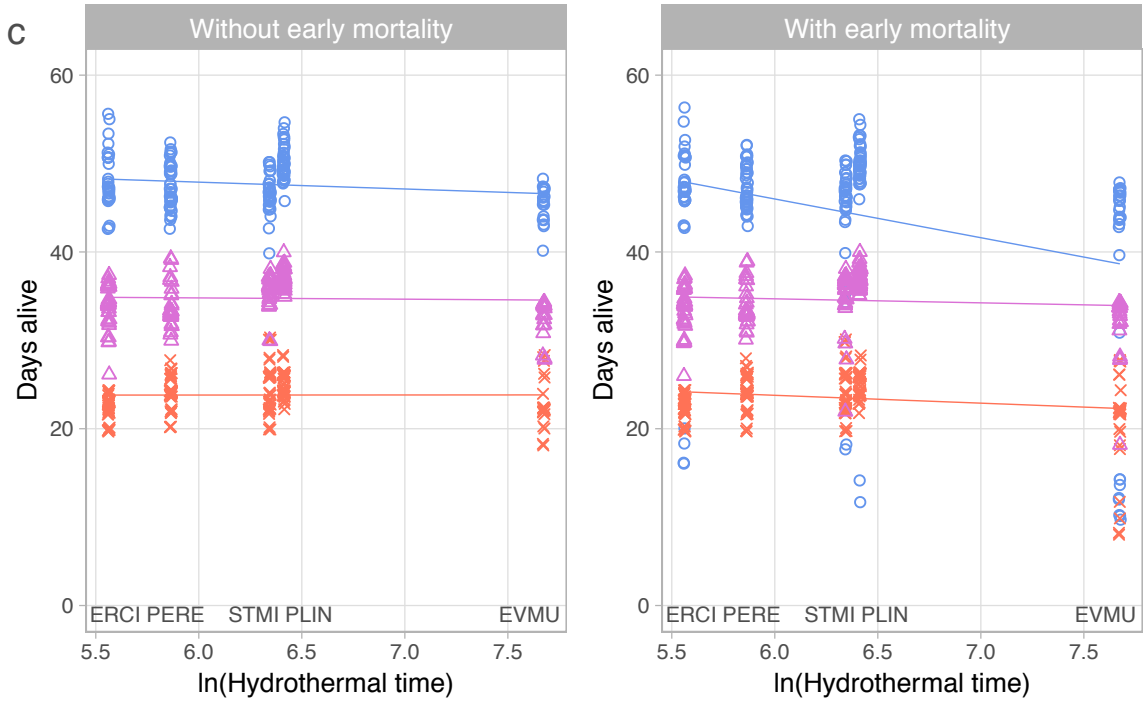
Figure 2. Cumulative proportion of deaths as a function of soil moisture loss at death. Solid, dashed and dotted lines represent plants in the cold (11 °C average temperature), medium (16 °C average temperature), hot growth chambers (21 °C average temperature), respectively. The x-axis is soil moisture, normalized to range from 0 (wettest soil) and 1 (driest soil), at which any plant died in the experiment. The y-axis shows cumulative proportion of deaths at different temperatures for each species (a) and for different species at each temperature (b).

Figure 3. Proportion of seedlings that died between their germination census and the following census, usually about a month later, averaged over 30 years of field data. Germination cohorts are grouped into early (September, October), mid (November), and late (December, January) periods within the germination season. Species are ordered from lowest to highest average days alive in the growth chamber experiment.

Figure 4. Growth rates and specific leaf area for plants grown under experimental drought at three growth chamber temperatures. Circles, triangles, and x's are individuals plants (a) or pot means (b) for seedlings in the cold (11 °C average temperature), medium (16 °C average temperature), and hot growth chamber (21 °C average temperature), respectively. Solid, dashed, and dotted lines denote regressions within the cold, medium, and hot growth chambers, respectively. Species' changes in mass over time are presented in the left graphs. The quadratic regression of log dry mass against age indicates a general decline in growth rate as water becomes scarce (a). The right graphs show changes in specific leaf area as plants grow represented by the slope of $\ln(\text{leaf area})$ as a function of $\ln(\text{above ground dry mass})$ (b).

Figure 1





○ 11°C △ 16°C × 21°C

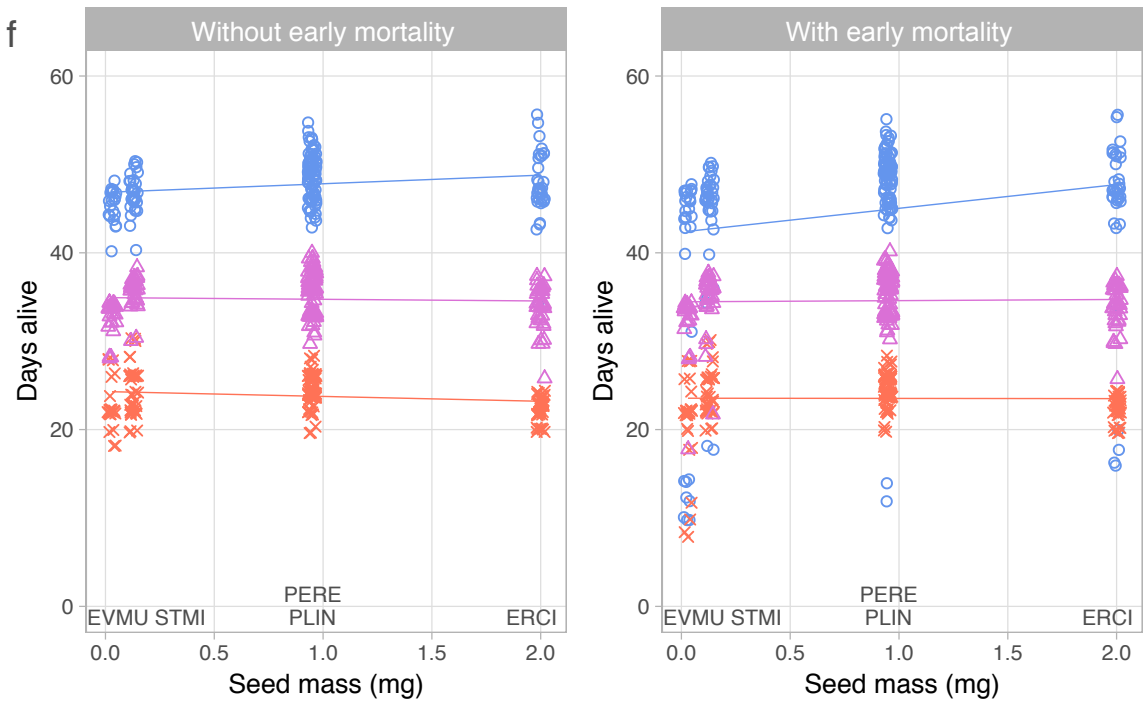
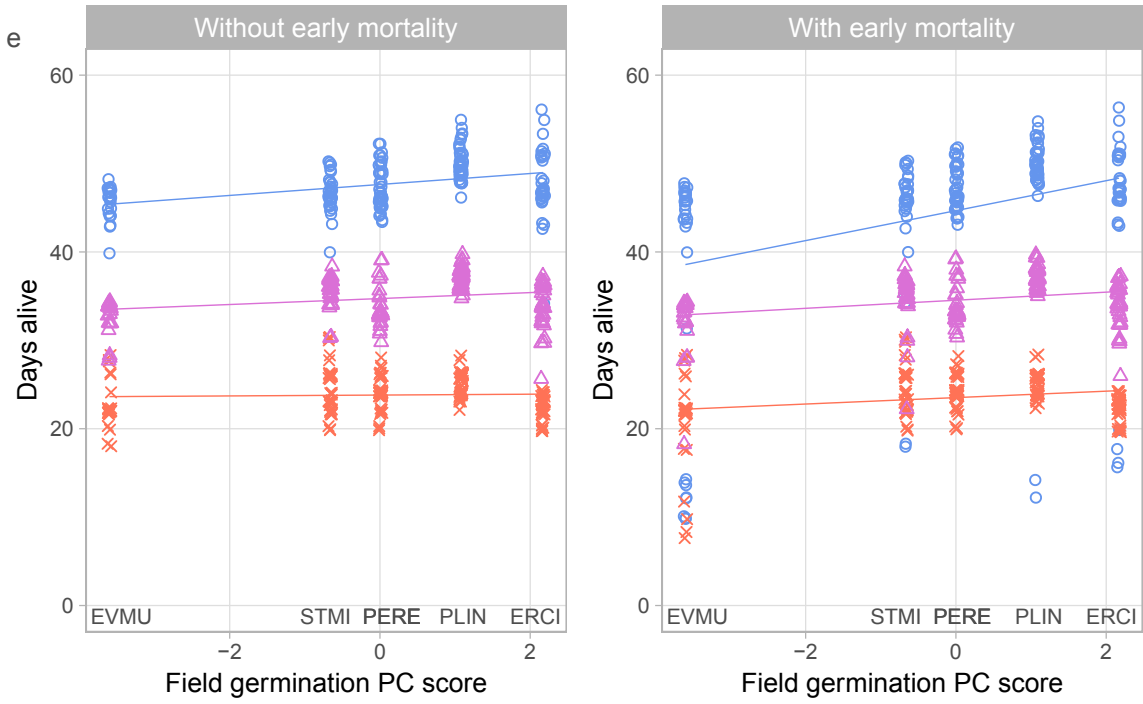
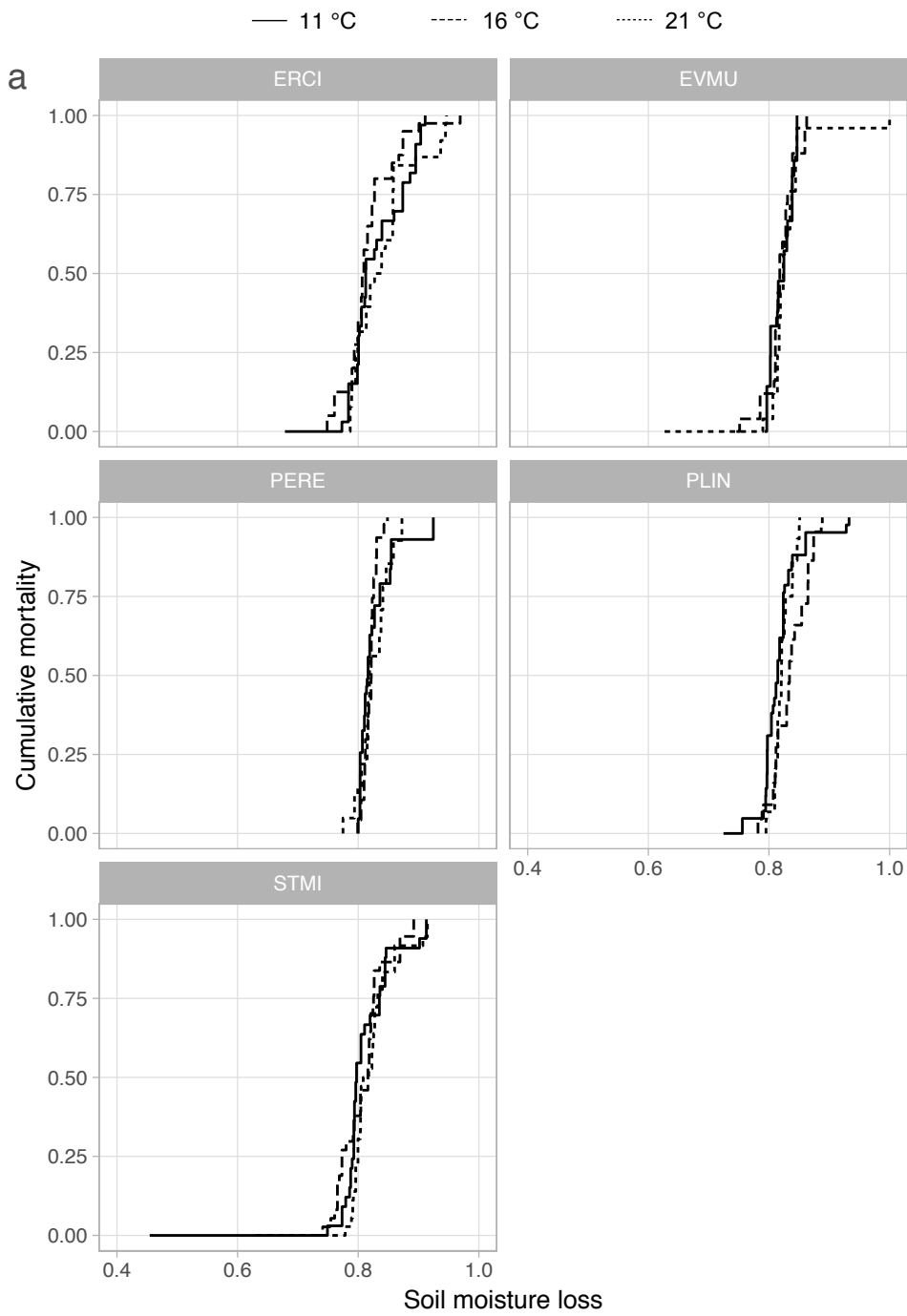


Figure 2



— ERCI ···· EVMU -·-· PERE - - PLIN ···· STMI

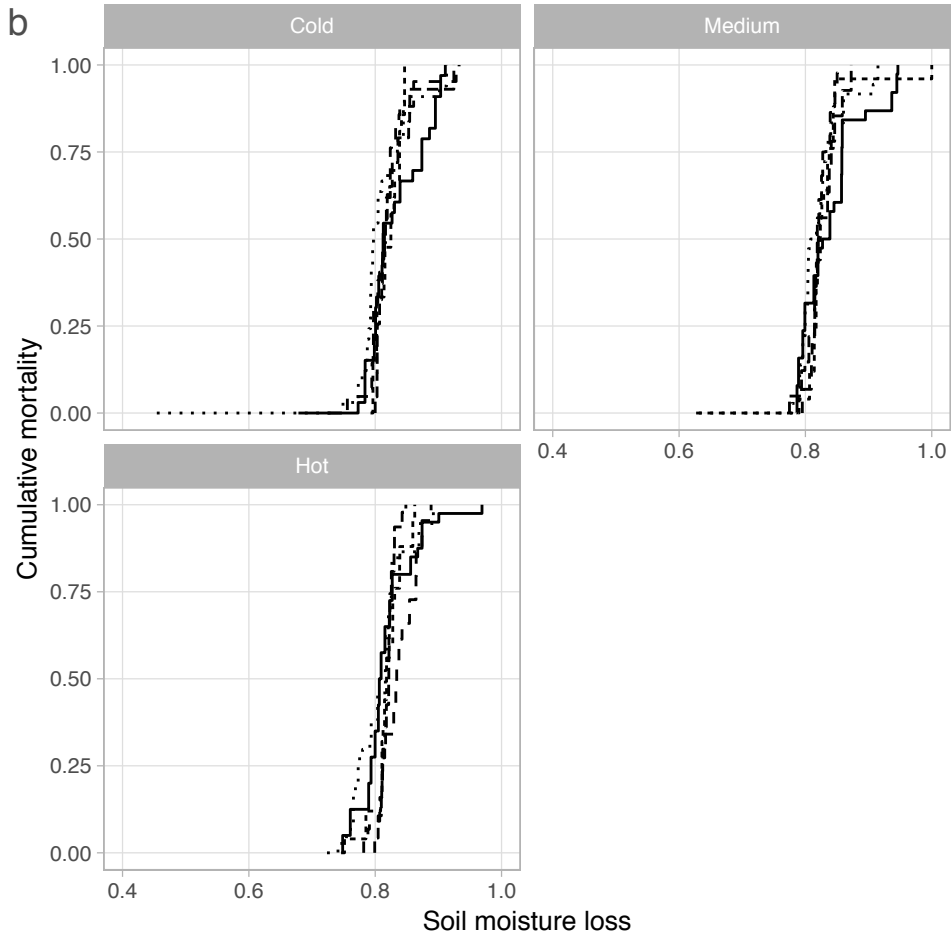


Figure 3

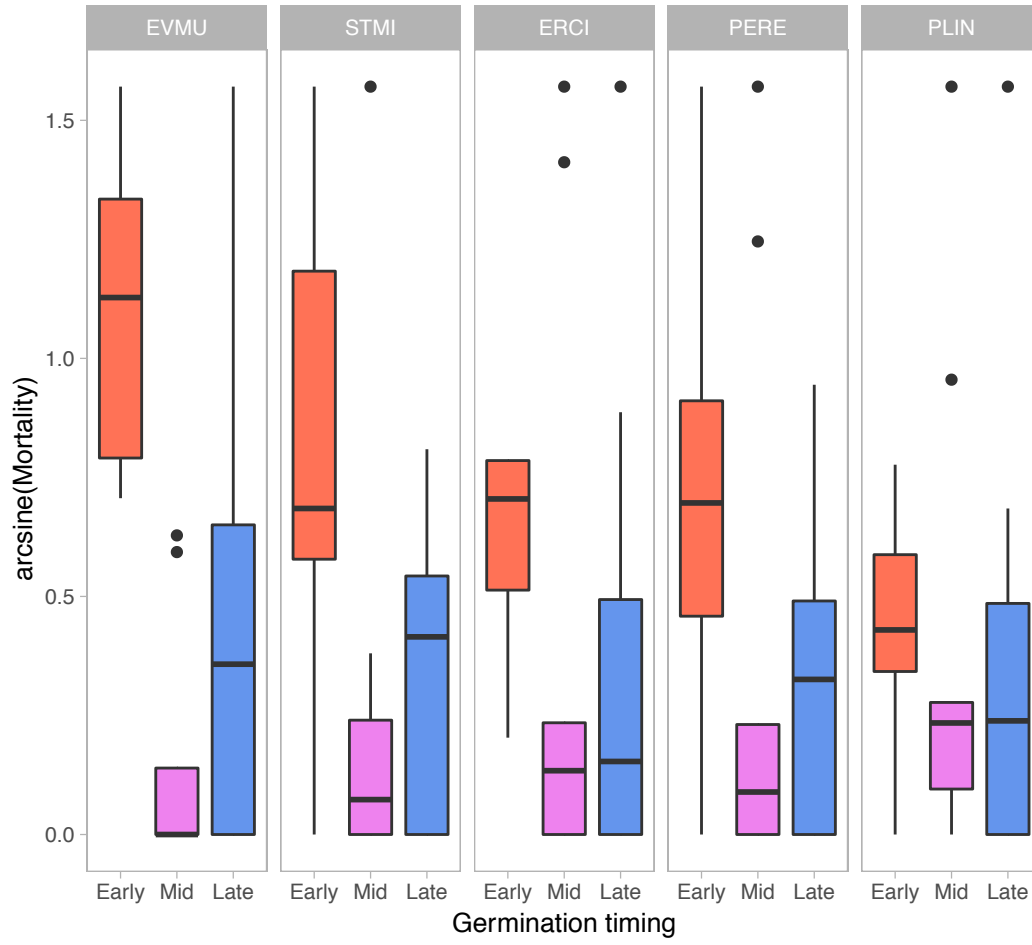


Figure 4

