Clinal variation in drought resistance shapes past population declines and future management of a threatened plant

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Abstract. Predicting vegetation responses to increased future drought is challenging, owing to the complex interaction of multiple factors influencing both plant drought resistance and local climatic conditions, each of which may be subject to spatial and temporal heterogeneity. We conducted a detailed study of potential mechanisms underlying an elevational gradient in mortality that has characterized recent population declines of a threatened alpine plant, the Haleakalā silversword (Argyroxiphium sandwicense subsp. macrocephalum). We used a pair of greenhouse experiments staged at high and low elevations to test the influences of plasticity (to contrasting soil water availability and atmospheric conditions) and heredity (different seed source elevations) on the development of functional traits associated with drought resistance and on performance in a terminal drought. We then out-planted a subset of plants into three common-garden plots near the bottom, middle, and top of the silversword elevational range, and tracked growth and survival over 3.5 yr. A range of morphological traits (root and leaf mass fractions, specific leaf area, leaf area ratio, and root length per shoot mass) exhibited either heritable variation or plasticity or both. Among physiological traits measured, only water use efficiency exhibited a plastic treatment response. Survival of out-plants was influenced during the first year by a home site advantage, and by the water treatment imposed in the greenhouses, with high water plants dying more often than low water plants. These effects subsequently dissipated, being replaced over the final 1.5 yr by a strengthening outplant site effect: survival was positively associated with site elevation, matching the pattern of mortality observed in the wild population. The balance of information suggests this pattern stems from lower elevation plants being less drought resistant than higher elevation plants, owing principally to plasticity, and thus suffering greater mortality during recent dry seasons. The pattern may be enhanced by stronger deviations from typical climatic conditions at lower elevations. Our findings suggest that future management should focus on climatically suitable habitat rather than advantageous genetic ecotypes. More broadly, they provide evidence that clinal variation can be important to consider when modeling future vegetation responses to climate change.

Key words: adaptation; Argyroxiphium; climate change; drought; functional traits; hydraulic strategy; silversword.

INTRODUCTION

Changes in regional patterns of precipitation and soil water status resulting from global climate change are likely to place severe stress on plant populations, species, and communities, particularly those that experience more frequent or more intense drought (Allen and Breshears 1998, Allen et al. 2010). Accurately predicting how vegetation will respond to water shortage across the landscape presents significant challenges, however, as such responses are dependent on a range of factors that interact with one another, often at different temporal and spatial scales. For example, regional changes in precipitation may affect plants heterogeneously at smaller scales as a result of local topographic, edaphic, or atmospheric features, and these changes may not covary uniformly with those of other climatic drivers such as temperature (Dobrowski et al. 2013, VanDerWal et al. 2013). Similarly, differences among plant species in their strategies for confronting water limitation will likely lead...
to variable responses within and among plant communities (Engelbrecht and Kursar 2003, Markestein and Poorter 2009, West et al. 2012). Even intraspecific variation in drought resistance (e.g., Abrams and Kubiske 1990, Zhang et al. 2011, Vasques et al. 2013) could produce complex spatial responses to changing climate for a given species. Moreover, such intraspecific variation can arise through both plastic trait development and heritable trait selection (Byars et al. 2007, Premoli and Brewer 2007, Kim and Donohue 2011), which operate on different time scales and may therefore further complicate patterns of response. This complexity is likely to be understood only through detailed empirical studies, which may then be used to inform and refine efforts to model broader vegetation responses to climate change (e.g., Thuiller et al. 2005, Fortini et al. 2013), and to develop management strategies for affected species.

The Haleakalā silversword (ʻiʻihihina; *Argyroxiphium sandwicense* subsp. *macrocephalum* (A. Gray) Meyrat) represents an excellent case in point. This tropical alpine rosette plant endemic to the island of Maui, Hawai‘i, is recognized locally and globally as a species of high conservation value, and is federally listed as a threatened species in the United States. Owing to its ecological and cultural importance and protected status, an abundance of ecological information has been collected for this species, which has revealed that its population has undergone a substantial climate-associated decline in recent decades, principally in the lower portion of its elevational range (Krushelnycky et al. 2013, 2016). Although it has been hypothesized that the lower elevation limit of Haleakalā silverswords (hereafter, silverswords) could be controlled in part by damage to the apical meristem from high temperatures (Melcher et al. 1994), the available demographic and climate data suggest that recent mortality has been driven more by drier conditions than by increasing temperature (Krushelnycky et al. 2013, 2016). The causes of the elevational pattern in mortality are therefore likely more complex than a simple upslope tracking of temperature and may involve interactions between changing climate gradients and local variation in drought resistance (Krushelnycky et al. 2016). Clarifying the drivers of population decline is central to this species’ conservation, while also contributing to the broader goal of understanding the responses of rare plant populations to a changing climate.

Silverswords grow from approximately 2,150 m elevation to the summit of Haleakalā volcano (3,050 m) in alpine desert characterized by very sparse vegetative cover and the predominance of xerophytic species (Leuschner and Schulte 1991). However, annual rainfall across the current silversword range is high relative to most arid regions, at ~1,000–1,350 mm/yr over the period of 1978–2007 (Giambelluca et al. 2013). Soil water limitation is therefore thought to result in part from high potential evapotranspiration in the region (Price et al. 2012), as well as from the highly porous nature of the young volcanic surface soils within Haleakalā Crater, which leads to rapid infiltration and low water holding capacity (Leuschner and Schulte 1991, Pérez 2001). Silverswords grow mainly on cinder cones and other cinder deposits overlaying parent material that ranges in age from approximately 1,500–30,000 yr, although some plants occur on parent material >140,000 yr old on the rims of the crater (Sherrod et al. 2007).

Körner (1999) cautioned that alpine plants typically do not experience frequent water stress despite the often barren appearances of their habitats because subsurface soil moisture levels are commonly ample and because alpine plants are well-adapted to local conditions. Silverswords appear to be highly adapted to their environment (Robichaux et al. 1990), and take advantage of favorable microsites such as nurse rocks that improve soil moisture and temperature status and increase seedling survival (Pérez 2015, 2017). Nevertheless, a ~60% decline in the silversword population size in recent decades has been strongly tied to reduced rainfall, which has coincided with other atmospheric changes such as higher solar radiation and vapor pressure deficit that appeared to result from an upward shift in the incidence of the trade wind inversion (TWI) around 1990 (Krushelnycky et al. 2013, 2016, Longman et al. 2015a). The TWI typically prevents the vertical expansion of clouds above roughly 2,200 m elevation on Haleakalā, creating drier atmospheric conditions higher on the mountain throughout most of the silversword range. Haleakalā’s pattern of declining precipitation and relative humidity with increasing elevation on upper slopes (Longman et al. 2015b) contrasts with many temperate alpine environments (Körner 1999). Furthermore, because ~70% of the annual rainfall within silversword habitat occurs during the winter wet season (November through April) (Krushelnycky et al. 2013), water stress experienced during the dry season (May through October) is strengthened by warmer summer temperatures. Future warmer and drier conditions in this alpine region may therefore make plants particularly vulnerable to water stress, as has been predicted for other summer-dry, winter-wet, Mediterranean-type ecosystems (West et al. 2012).

While rainfall has generally decreased across most of Hawai‘i over the past century, and especially at high elevations (Frazier and Giambelluca 2017), a continued drying pattern on upper Haleakalā is uncertain. Two different methods for downscaling global circulation models have resulted in different predictions for precipitation patterns within silversword habitat. A statistical downscaling effort projected declining rainfall year-round that will be especially pronounced in the dry season on upper Haleakalā by the mid-21st century (Elison Timm et al. 2015). By comparison, a dynamical downscaling effort projected somewhat wetter wet seasons and generally unchanging rainfall during dry seasons within silversword habitat by the late 21st century (Zhang et al. 2016). Also relevant are future projections that the TWI in Hawai‘i will decrease in elevation (Lauer et al. 2013, but see Zhang et al. 2016) and increase in frequency (Zhang et al. 2016), both of which would result in drier
conditions within silversword habitat. Given these future climate possibilities, a better understanding of silversword drought resistance will be critical for managing its continued persistence.

Although a strong climate link with the recent silversword population decline has been demonstrated (Krushelnycky et al. 2013, 2016), the specific mechanism(s) underlying increased mortality at lower elevations remains unresolved. One possible explanation for higher mortality at lower elevations is that warmer temperatures create larger soil water deficits during dry periods through greater evapotranspiration. Greater water stress has been identified as a factor likely contributing to higher mortality of trees in lower elevation or otherwise warmer regions during drought at a number of locations (Allen et al. 2010). Another possibility is that the inverse relationship of rainfall with elevation on upper Haleakalā may result in significant geographic variation in drought resistance within the silversword population, with plants growing at higher elevations being more resistant than those growing at lower elevations. During severe dry periods, low-elevation plants may consequently suffer greater mortality, especially if the deviation from normal conditions is greater at low elevations. Intraspecific genetic variation in plant traits or ecotypes associated with drought resistance has been documented in a wide range of plants, including grasses (Byars et al. 2007, Zhang et al. 2011), herbs and trees (Abrams and Kubiske 1990, Premoli and Brewer 2007, Kim and Donohue 2011), and even tropical alpine rosettes (Baruch 1979). Likewise, phenotypic plasticity of such traits relative to differential water availability is a common phenomenon in plants (e.g., Bell and Sultan 1999, Padilla et al. 2009, Olmo et al. 2014).

The aforementioned mechanisms are not mutually exclusive, and multiple mechanisms may operate simultaneously. Yet, each has different implications for population responses to potentially warmer and drier future conditions, and for associated management options. Heritable variation in drought resistance across the silversword population, if present, might shift in frequency too slowly via natural selection to mitigate the effects of anthropogenic climate change (Jump and Peñuelas 2005), but could be used to increase overall resistance through strategic cross-breeding or translocation of resistant genotypes. Variation in drought resistance that results entirely from plasticity, in contrast, would preclude the benefits of such human-assisted outcrossing, but might provide a natural buffer against a changing climate, depending on the rate, intensity, and spatial pattern of the changes (Valladares et al. 2014). Importantly, an absence of substantial intraspecific variation in drought resistance would suggest that the dynamics of future climate forcing alone will dictate population response patterns. In this case, identifying the most suitable future habitat locations, and out-planting individuals to them, may be the most effective management strategy (e.g., Marrero-Gómez et al. 2007).

We undertook a study to clarify the mechanisms associated with the recent population declines of Haleakalā silverswords, with the explicit goal of identifying possible conservation strategies in the event that recent drying patterns continue into the future. We employed two approaches, controlled greenhouse trials and common-garden out-planting, to understand sources of variation in drought resistance in young silversword plants, which is the life stage that has contributed most to recent mortality in the field (Krushelnycky et al. 2013). The greenhouse component implemented a full-factorial test of the influences of growth elevation (plasticity induced by temperature and evaporative demand), soil water availability (plasticity induced by the simulated rainfall gradient), and seed source elevation (heritable genetic differentiation) on the development of functional traits associated with drought resistance, and on subsequent performance in an imposed terminal drought. This component also provided insight into the silversword’s hydraulic strategy for persisting in its environment via drought resistance. We use drought resistance here sensu Öpik and Rolfe (2005) as an inclusive term for various strategies to deal with water stress, including drought tolerance and drought avoidance. Subsequently, we out-planted a subset of the greenhouse plants into three plots located near the bottom, middle and top of the silversword elevational range, and tracked plant growth and survival over the course of three and a half years. Together, the greenhouse and field study components provided important complementary information about how silverswords respond to environmental variation and stress, informing expectations of future population trends and management possibilities under a changing climate.

**Materials and Methods**

**Study site and species**

The Haleakalā silversword is a federally listed threatened taxon in the family Asteraceae that occurs only on East Maui, Hawai‘i. The Haleakalā silversword is a long-lived (estimated 20–90 yr; Rundel and Witter 1994), monocarpic, self-incompatible, acaulescent rosette plant that today grows on the largely barren cinder cones, cinder flats, and rocky cliffs in a broad geographic area (roughly 2,300 ha) spanning the central to western portions of Haleakalā Crater up to the summit. Silversword distributions within the total range on upper Haleakalā volcano are clumped, with distinct aggregations often separated by large areas devoid of individuals. The most recent estimate of the total population size was roughly 40,000 plants in 2013 (Krushelnycky et al. 2016).

**Experimental design**

A greenhouse study was paired with a common-garden out-planting study to test the effects of growth elevation, soil moisture availability and seed source
elevation on seedling development and performance during drought. Seeds for all plants in both experiments were collected in September–October 2012 from four maternal plants in each of three areas spanning the elevational range of silverswords: low elevation (Silversword Loop, 2,185 m elevation, 1,280–1,320 mm mean annual precipitation [MAP]), mid-elevation (Ke‘onehe‘ehe‘e Trail, 2,480 m elevation, ~1,125 mm MAP), and high elevation (West Rim and Pa Ka‘a‘ao, 2,895 m, ~1,050 mm MAP). MAP estimates were calculated over the period 1978–2007 (Giambelluca et al. 2013). Unfertilized achenes were discarded and fertilized seeds were stored in refrigeration until 9–10 September 2013, when single seeds were sowed into pots (1.6 L, 24.1 cm high × 10.2 cm wide) filled with 1:1:1 fine cinder:perlite:vermiculite soil medium. Each pot received one level one-quarter teaspoon (average of 0.9 g) of slow-release fertilizer pellets (Nutricote Total 13-13-13, Type 180; Chisso-Asahi Fertilizer, Tokyo, Japan), and pots were watered twice daily for 15 minutes by overhead sprinklers in a large greenhouse located at 2,070 m elevation until they were transferred to a pair of study greenhouses.

Seedlings originating from each of the 12 maternal plants were randomly assigned to the 12 treatment groups arising from the fully crossed factorial design of the three main effects of interest: three source elevations × two water treatments × two growth elevations. The 12 treatment groups were further nested in four fate categories: growth, drought, out-plant, and extras. Growth plants (n = 144) were harvested for morphometric analysis at the end of the growth period; drought plants (n = 288) were subjected to terminal drought at the end of the growth period to assess performance under water stress; out-plants (n = 144) were planted in experimental plots at the end of the growth period to assess survival in the field; and extras (n = 96) were retained to replace seedlings (from the same maternal plant) that perished before the end of the growth phase. We prepared an additional 15 pots filled only with soil for each greenhouse to track rates of soil water evaporation.

On 5 November 2013, we initiated the experiment by transferring the seedlings to two study greenhouses (Appendix S1: Figs. S1–S3) located at 2,135 m and 3,000 m elevation, the lower and upper bounds of the silversword range, according to their prior assignments for the growth elevation factor (n = 336 in each greenhouse). Air temperature and relative humidity at pot level were recorded every 3 minutes with a datalogger (HOBO U23-002; Onset Computer, Bourne, Massachusetts, USA). Vapor pressure deficit (VPD) in the greenhouses was calculated from hourly air temperature and relative humidity averages according to Lowe (1976). Pots were randomly arranged in trays holding 12 pots each, and tray positions within each greenhouse were randomly shuffled every two weeks.

Seedlings were watered once weekly using methods modified from Canham et al. (1996) and Sack (2004). All seedlings were watered to field capacity at the outset of the experiment, and seedlings assigned to the low soil moisture water treatment were given less water each week until the mean gravimetric soil water content (SWC) in each greenhouse, after watering, attained approximately 50–60% of field capacity (PFC). Because we held the weekly watering volume constant between greenhouses (for each water treatment level), and because evapotranspiration rate varied somewhat between greenhouses, mean SWC differed slightly between greenhouses, but generally stayed within the weekly rewatering target range throughout most of the growth period (Fig. 1). In an analogous fashion, we watered the high soil moisture water treatment plants so as to maintain the weekly rewatering mean SWC in each greenhouse at a target of 90–100 PFC (Fig. 1). We also measured the maximum rosette diameter of one-half of all plants weekly to track aboveground growth rates during the growth period (Fig. 1), which terminated on 6 June 2014, 31 weeks after study initiation.

Seedling functional traits

On 11 June 2014, we began harvesting seedlings in the growth fate category to measure morphological and physiological traits. This process took several months, and seedlings continued to be watered according to the methods above until their harvest date, with the timing of harvest evenly distributed among treatment groups. For all plants, we separated the aboveground rosette, removed and photographed all leaves for leaf area calculation using ImageJ software (Rasband 1997–2014), and weighed the leaf and stem biomass after drying at 60°C for 48 h. Roots were carefully separated from the soil, pressed flat in a water bath, and total root length (R) was estimated by counting the number of root intersections with a 2 × 2 cm grid using the formula $R = (\pi \times N \times A)/2H$, where $N$ is the number of intersections, $A$ is the area of the grid, and $H$ is the summed length of all lines in the grid (Böhm 1979, Poorter and Marksteijn 2008). Roots were then dried at 60°C for 48 h and weighed. Using these measurements, we calculated the following morphometric traits that have been shown to be related to variation in drought resistance and water economy (Sack 2004, Cornwell et al. 2007, Poorter and Marksteijn 2008, Marksteijn and Poorter 2009): root mass fraction (RMF, root dry mass/plant dry mass, unitless), leaf mass fraction (LMF, leaf dry mass/plant dry mass, unitless), specific leaf area (SLA, total leaf area/leaf dry mass, units cm$^2$/g), leaf area ratio (LAR, total leaf area/plant dry mass, units cm$^2$/g), specific root length (SRL, total root length/root dry mass, units m/g), root length per shoot mass (RLSM, total root length/shoot dry mass, units m/g), and root length per leaf area (RLLA, total root length/total leaf area, units m/cm$^2$). We also calculated relative growth rate (RGR, units g/g/d) according to the formula $\text{RGR} = (\ln(\text{final dry mass}) - \ln(\text{initial dry mass}))/\text{growth period}$ (Sack 2004).
For five seedlings in each of the 12 treatment groups, we determined the shoot water potential at the turgor loss point (Ψ_{tp}, units MPa) and leaf capacitance (C, units MPa^{−1}) before making the measurements described above. Seedlings were kept in saturated soil overnight, then the entire shoot was separated from the roots and the stem base was submerged in distilled water for 45–60 minutes to ensure full saturation. Pressure–volume curves were then generated following the procedures of Koide et al. (1989), with the entire shoot being inserted in a pressure chamber (model 600; PMS Instrument, Albany, Oregon, USA). Because capacitance typically declined rapidly below water potentials of approximately −0.5 MPa (Fig. 2), we calculated average C above this threshold as ΔR/ΔΨ, where R is the relative water content (Robichaux and Morse 1990).

For four seedlings in each of the 12 treatment groups, we measured gas exchange rates on two occasions (between 08:00 and 11:30) between two weekly watering events near the end of the growth period (21–27 May 14), using a LI-6400XT photosynthesis system (LI-COR Biosciences, Lincoln, Nebraska, USA). The leaf chamber was set to a reference CO₂ concentration of 400 ppm (equals partial pressures of CO₂ of 31.3 and 28.1 Pa in lower and upper greenhouses, respectively), PAR level of 1,000 l mol/m²/s, 17°C, and RH of 50%. The two measurements of net photosynthetic rate (A_{net}, units μmol CO₂·m⁻²·s⁻¹), stomatal conductance
occurred. For analyses, we excluded measurements after SWC fell below 5%, at which point little or no gas exchange was observed. Each pot was also weighed at the time of mortality. Three seedlings from each treatment group in each greenhouse were randomly selected to be measured in each greenhouse between 08:00 and 11:30 every MWF during the drought, starting on 27 June (4 d after drought initiation), and continuing until seedlings died. LI-COR settings were the same as indicated above. Each pot was also weighed at the time of gas exchange measurement to determine current SWC. For analyses, we excluded measurements after SWC fell below 5%, at which point little or no gas exchange occurred.

**Out-planting experiment**

Near the end of the growth period, the 144 seedlings in the out-plant fate category (n = 72 in each greenhouse) were randomly assigned, within treatment groups, to one of three out-planting plots, such that each plot received four plants from each of the 12 treatment groups (n = 48 plants total per plot). The plots were located adjacent to existing natural populations at three elevations (2,185, 2,425, 2,900 m; Appendix S1: Figs. S4–S6). Long-term (1978–2007) MAP is estimated to be 1,282 mm at the low-elevation plot, 1,129 mm at the mid-elevation plot, and 1,082 mm at the high-elevation plot (Giambelluca et al. 2013). At each plot, we planted seedlings 1.5 m apart in four to seven lines with seedling position randomly assigned, on 28–30 May 2014. Each seedling received 1 L of water immediately after planting, with no subsequent watering. Maximum rosette diameter and survival status were measured approximately quarterly after planting until 2 November 2017, the end of the fourth dry season post-planting (41 months). Because the square of rosette diameter was correlated with total biomass both early in the growth phase (r = 0.888, P < 0.001, Appendix S1) and at the end of the growth phase (r = 0.800, P < 0.001), we calculated RGR over the total monitoring period (for surviving out-plants) as (ln([final diameter]2) − ln([initial diameter]2))/growth period [in days]), with change in rosette diameter (cm2 cm−2 d−1) estimating change in dry mass (g g−1 d−1).

Weather conditions at each plot were monitored with nearby weather stations that recorded rainfall, air temperature, relative humidity, soil moisture, soil temperature at 5 cm depth, and leaf wetness at 10-minute intervals (H21 datalogger [Onset Computer] with S-RGB-M002 rain gauge, S-THB-M002 air temperature/RH sensor, S-SMA-M005 ECH2O 254 × 32 mm soil moisture sensor, S-TMB-M006 soil temperature sensor, and S-LWA-M003 leaf wetness sensor [Decagon Devices, Pullman, Washington, USA]. VPD was calculated according to Lowe (1976), and we calculated the percentage of days that were rainless because this metric was previously found to correlate with silversword demographic patterns (Krushelnycky et al. 2013).

**Analyses**

Variation in plant traits was assessed with linear mixed models (LMMs) that fitted growth elevation, water treatment, source elevation, and their interactions as fixed effects, and maternal plant as a random effect, to each response. We also conducted a Principal Components Analysis (PCA) on the seven morphological traits calculated (RMF, LMF, SLA, LAR, SRL, RLSM, RLLA), and used the resultant PCA axis 1 as a composite response in the LMM. Variation in plant longevity and SWC at time of mortality during the terminal greenhouse drought were assessed with LMMs that fitted growth elevation, drought elevation, water treatment, source elevation, and their interactions as fixed effects, rosette diameter and the total amount of soil water in the pot at the outset of the drought (maximum water) as

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**Fig. 2.** Example pressure–volume curves from four greenhouse plants, indicating a slow decline in water potential with decreasing relative water content, owing to leaf capacitance, until water potential \( \Psi \) reaches about −0.5 MPa.

\[ g_s \text{ units mol H}_2\text{O m}^{-2} \text{s}^{-1}, \text{transpiration } (E, \text{ units mmol H}_2\text{O m}^{-2} \text{s}^{-1}) \text{ and water use efficiency (WUE, calculated as } A_{net}/(E \times 10), \text{ units } \mu \text{mol CO}_2/\text{cmol H}_2\text{O}) \text{ for each plant were averaged for analysis.} \]
fixed covariates, and maternal plant as a random effect. Variation in gas exchange rates ($A_{net}$, $g_s$, $E$, WUE) during the drought were assessed with LMMs that fitted growth elevation, drought elevation, weather treatment, source elevation and their interactions as fixed effects, log(SWC) at the time of measurement as a fixed covariate, and seedling identity as a random effect.

Variation in RGR of the surviving out-plants was assessed with a LMM that fitted growth elevation, water treatment, source elevation, out-plant site, and their interactions as fixed effects, initial rosette diameter as a fixed covariate, and maternal plant as a random effect. Because survival patterns changed over time, we assessed survival probabilities from the moment of out-planting to the end of each season (starting with the first dry season at 5 months post-planting and subsequently every 6 months through the end of the fourth dry season at 41 months), with generalized linear models (GLMs) that fit plant survival as the binary response, using the binomial distribution and logit link function. For each time increment, we fit the four main fixed explanatory factors of interest (growth elevation, water treatment, source elevation, out-plant site), home site (yes, no) to indicate whether plants were out-planted near their seed source locations, maternal plant as a fixed effect, and average rosette diameter as a fixed covariate.

We analyzed weather associations with probability of out-plant mortality by tabulating the survival status of each plant during each quarterly monitoring interval throughout the study, and fitting this response to weather patterns at each site with generalized linear mixed models (GLMMs) using the binomial distribution and logit link function. We filled missing data caused by plant mortality with imputed data (Twisk 2003; an identical analysis without imputed data yielded very similar results). Four of eight weather variables considered (relative humidity, leaf wetness, soil temperature, percentage of rainless days) were strongly correlated ($r > 0.75$) with other weather variables, and were excluded from analysis. Averages for the remaining four variables (daily rainfall, air temperature, soil moisture, VPD) were calculated for 90 d preceding each monitoring date at the three out-planting sites to measure their influences on rates of mortality. Evaluation of GLMMs was done within an information-theoretic and multi-model inference framework (Burnham and Anderson 2002): models including all combinations of the four fixed weather variables and all of their two-way interactions (plus rosette diameter as a size covariate and plant identity as a random effect) were computed for each out-planting site, resulting in a total of 20 models for each site. Models within each set were ranked using AIC, removing all models with $\Delta$AIC $> 2$, and subsequently removing more complex versions of nested models that differed by only one parameter and had similar log-likelihood values to simpler models (Burnham and Anderson 2002, Pasinelli et al. 2016). Finally, we averaged the remaining models for each site to obtain weighted-average parameter coefficients, using the conditional averaging method (Grueber et al. 2011).

GLMMs were run with the lme4 package (Bates et al. 2016) and model averaging was done with the MuMIn package (Bartoń 2015) in R (R Core Development Team 2017). All other analyses were performed in SAS JMP Pro 13.1.0. Additional details of study design, methods, and analysis are provided in Appendix S1, Supplementary Methods and Materials.

RESULTS

Growth and drought conditions in the greenhouses

Air temperatures were substantially higher in the low-elevation greenhouse than in the high-elevation greenhouse during the growth period (Appendix S2: Fig. S1; hourly values 13.42°C ± 0.08°C vs. 9.90°C ± 0.08°C [mean ± SE], respectively). However, evaporative demand, measured by VPD, was similar between the greenhouses for most of the growth period (Appendix S2: Fig. S1), and mean values over the period were nearly identical (6.93 ± 0.09 hPa in low greenhouse vs. 7.03 ± 0.08 hPa in high greenhouse). This resulted from higher relative humidity at the lower elevation location, owing to ground-level clouds that commonly formed at the base of the TWI. The latter weather pattern became especially prominent starting around May, resulting in greater evaporative demand in the upper greenhouse for much of the remaining spring and summer, including most of the drought phase of the experiment (Appendix S2: Fig. S1; drought period, 9.75 ± 0.09 hPa in low greenhouse vs. 10.68 ± 0.08 hPa in high greenhouse). This occurred despite cooler temperatures in the upper greenhouse during the drought period (Appendix S2: Fig. S1; drought period, 17.11 ± 0.08°C in low greenhouse vs. 14.39 ± 0.08°C in high greenhouse). The higher VPD in the upper greenhouse produced higher rates of soil water evaporation in unsowed pots compared to the lower greenhouse during the first six weeks of the drought (Appendix S2: Fig. S2).

Greenhouse seedling functional traits

Many of the morphological and physiological traits were significantly inter-correlated (Appendix S2: Table S1). Most of these traits were also significantly correlated with seedling biomass (Appendix S2: Table S1), potentially indicating important ontogenetic effects on seedling biomass. When adjusting for seedling size by including biomass as a covariate, all three main factors of interest (growth elevation, water treatment, and source elevation) had significant effects on variation of at least some of the functional traits (Table 1), and generally influenced them in the expected directions. High growth elevation significantly decreased SLA and LAR and increased WUE, suggesting enhanced drought resistance relative to low growth elevation (Table 1, Fig. 3). The low water treatment
### Table 1. Results of LMMs testing the roles of growth elevation, water treatment and source elevation, and their interactions, on morphological and physiological traits

<table>
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<th>Trait</th>
<th>Growth elevation (GE)</th>
<th>Water treatment (WT)</th>
<th>Source Elevation (SE)</th>
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<th>GE*SE</th>
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<td>0.704</td>
<td>2,123</td>
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</table>

Notes: Maternal plant was included as a random factor, and seedling biomass was included as a covariate in all models except that for RGR. Significant effects ($P < 0.05$) highlighted in bold.
FIG. 3. Traits that exhibited significant variation with respect to the three main treatment factors when controlling for seedling size (with the exception of RGR). Values are least squares means (±SE) from the mixed models shown in Table 1. Means sharing the same letter within each panel are not significantly different (Tukey HSD test, α = 0.05). Units for traits are provided in Methods. RGR, relative growth rate; RMF, root mass fraction; LMF, leaf mass fraction; SLA, specific leaf area; LAR, leaf area ratio; RLSM, root length per shoot mass; WUE, water use efficiency; PCA1, principal components analysis axis 1.
increased RMF and RLSM and decreased LMF, suggesting enhanced drought resistance relative to the high water treatment (Table 1, Fig. 3). Low source elevation seedlings had lower RMF and higher LMF than seedlings from middle and high source elevations, suggesting weaker drought resistance in the low-elevation seedlings relative to the other two groups (Table 1, Fig. 3).

Physiological trait variation was weaker than morphological variation. Water potential at turgor loss point (Ψtp) was not significantly influenced by the treatment factors, although it exhibited nonsignificant variation in the expected direction in response to all three treatments: means were more negative at the high growth elevation, in the low water treatment, and at higher source elevations (Appendix S2: Fig. S3). Capacitance (C) exhibited little variation among treatment groups (Appendix S2: Fig. S3). Among the gas exchange rate parameters, only WUE was significantly affected by any of the treatment factors (growth elevation, Table 1, Fig. 3, Appendix S2: Fig. S3). The similar rates of \( \Delta_{net} \) at the two growth elevations suggest a plastic increase in photosynthetic capacity at high elevation (Bresson et al. 2009), where the partial pressure of CO2 is lower, because there was no difference in carbon assimilation rates tied to seed source elevation.

There were no significant interactions between the main effects for any of the traits measured (Table 1), indicating that traits influenced by multiple factors were affected approximately additively. RMF, for example, was maximized for high-elevation source plants grown under the low water treatment. This also indicates a lack of significant genetic variation in plasticity among source locations. Maternal plant identity accounted for little of the variation within the traits measured, with only SRL exhibiting significant variation among maternal plants (Table 1).

Axis 1 of the PCA ordination of the seven morphological traits explained 53.2% of the variance, and grouped traits that generally promote drought resistance (higher RMF, RLSM, RLLA) on the left side of the axis, and those that typically lessen drought resistance (higher LMF, LAR, SLA) on the right (Appendix S2: Fig. S4). SRL was also on the positive side of the axis, although it loaded more heavily onto Axis 2, which explained an additional 25.6% of variation. When PCA axis 1 was used as a composite response, low-water treatment and high-source-elevation plants had significantly lower scores than high-water treatment and low-source-elevation plants, respectively (Table 1, Fig. 3), suggesting enhanced drought resistance among the former groups.

High growth elevation plants also had lower axis 1 scores than low growth elevation plants, but this difference was only marginally significantly (Table 1).

RGR was significantly affected by all three treatment factors (Table 1, Fig. 3). The three factors again acted approximately additively, with RGR maximized for low-elevation-source plants grown at low elevation under the high water treatment. Differences in RGR have potential relevance to the interpretation of the three treatment effects because seedling biomass was significantly positively correlated with RMF and RLLA, and was significantly negatively correlated with LMF, SLA, LAR, SRL, and PCA axis 1 (Table 1). Most or all of these relationships suggest increasing drought resistance with increasing seedling size. Removal of the biomass covariate from the models therefore resulted in somewhat different results (Appendix S2: Table S2), including a few reversals in the directionality of the main treatment effects (Appendix S2: Figs. S5, S6). For example, both SLA and LAR were significantly higher for seedlings grown at low elevation on a size-adjusted basis, but were significantly higher for seedlings grown at high elevation when size was not accounted for. Trends in other traits, like RMF and RLSM, were similar regardless of whether size was considered.

**Greenhouse drought performance**

Seedling size had a very strong influence on longevity in the imposed terminal drought, dwarfing the effects of all other explanatory variables in the model (Table 2): larger plants died more quickly than smaller plants (Appendix S2: Fig. S7). Similarly, the amount of soil water in each pot at the outset of the drought (maximum water), which varied among pots by chance, exerted a significant effect and was positively associated with seedling longevity (Table 2, Appendix S2: Fig. S8). After adjusting for these two factors, several of the main factors of interest also influenced seedling longevity, although in less straightforward ways. The strongest of these effects resulted from growth elevation, drought elevation, and their interaction (Table 2, Fig. 4). Plants grown at low elevation lived approximately 15% longer when subjected to drought under low-elevation conditions compared to high-elevation conditions (45.3 ± 1.1 d vs. 39.5 ± 1.0 d; \( P < 0.05 \), Tukey HSD test), whereas drought elevation had little effect on longevity of plants that were grown at high elevation (Fig. 4). There was also an apparent effect of source elevation on longevity, but only in a three-way interaction with growth elevation and water treatment (Table 2). Specifically, seedlings sourced from high elevation lived significantly longer than seedlings sourced from middle elevation (45.1 ± 1.8 d vs. 35.4 ± 1.9 d; \( P < 0.05 \), Tukey HSD test), but only when the seedlings were grown at high elevation under the low water treatment. Longevities were not significantly different among any of the other combinations of these three factors (Tukey HSD test, \( \alpha = 0.05 \)).

Seedling size and maximum pot water volume had statistically significant but biologically very small effects on the SWC at the time of mortality (Table 2, Appendix S2: Fig. S9); no other factors had significant influence on the SWC at mortality (Table 2). All treatment groups therefore died at similarly low SWC of approximately 1.5–2.0% on average.
TABLE 2. Results of LMMs testing the roles of growth elevation, drought elevation, water treatment and source elevation, and their interactions, on metrics of seedling performance during terminal drought.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Longevity</th>
<th>SWC at mortality</th>
<th>n</th>
<th>A_{net}</th>
<th>m</th>
<th>E</th>
<th>WUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>P</td>
<td>df</td>
<td>F</td>
<td>P</td>
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<td>1,253</td>
<td>0.25 0.620</td>
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<tr>
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<td>0.93 0.337</td>
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<td></td>
<td>1,253</td>
<td>0.68 0.530</td>
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<tr>
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<td>0.93 0.337</td>
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<td>df</td>
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<td></td>
<td>1,253</td>
<td>0.68 0.530</td>
<td></td>
<td>1,49</td>
</tr>
</tbody>
</table>

Notes: Rosette diameter, max water and SWC were included as covariates, and maternal plant and seedling identity were random factors. Significant effects ($P < 0.05$) highlighted in bold.
As expected, rates of $A_{\text{net}}$, $g_s$, and $E$ declined as soil water was depleted during the drought. The log(SWC) was strongly and positively related to all three gas exchange parameters (Appendix S2: Fig S10), and explained the majority of variation in the models for these three responses (Table 2). After controlling for soil water status, there was little variation in $A_{\text{net}}$ tied to the other explanatory factors (Table 2). A significant interaction between growth elevation and drought elevation indicated that plants grown at low elevation tended to have higher rates of $A_{\text{net}}$ when experiencing drought at high elevation, while plants grown at high elevation exhibited the opposite trend (Fig 5); however, mean rates of $A_{\text{net}}$ were not significantly different among any of these four treatment group combinations (Tukey HSD test, $\alpha = 0.05$). The traits $g_s$, and $E$ were both significantly higher under low-elevation drought conditions (Table 2, Fig. 5), but were not significantly affected by growth elevation, water treatment, or source elevation (Table 2). As a result of lower rates of $E$ combined with similar rates of $A_{\text{net}}$, plants experiencing drought at high elevation exhibited higher WUE than plants experiencing drought at low elevation (Table 2, Fig 5). WUE was not significantly affected by the other main explanatory factors (Table 2).

**Climatic conditions at out-planting sites**

Rainfall was relatively similar across the three out-planting sites over the course of the study period (Fig. 6). Compared to prior years, dry season rainfall was substantially higher during most of the study period, due in part to prevailing ENSO conditions, which typically create wetter than normal dry seasons and drier than normal wet seasons in Hawai‘i (Chu and Chen 2005). Rainfall then dropped sharply in the final dry season of 2017 (Fig. 6). Soil moisture measured over the top 25 cm varied among sites much more than did rainfall (Fig. 6), apparently owing mainly to differences in physical properties of the upper soil layers (see Appendix S2), and likely did not accurately reflect among-site differences in total water availability. Within each site, however, soil moisture seasonal averages were surprisingly constant and relatively high over most of the study period, unlike in prior years when they fluctuated strongly between wet and dry seasons (Fig. 6). The high dry season rainfall in the first few years of the study prevented dry season declines in soil moisture until the final dry season, during which soil moisture was 9.0%, 10.9%, and 21.1% lower than the average of the three prior, post-out-planting dry seasons at the high, middle, and low-elevation sites, respectively. Air temperature displayed the expected inverse relationship with elevation, and higher average values in the dry seasons than the wet seasons (Fig. 6). VPD increased with elevation, but contrary to the few years prior to out-planting and to long-term patterns (Longman et al. 2015b), was higher in the wet seasons than the dry seasons during most of the study period (related again to ENSO; Fig. 6). In the final dry season, VPD was 17.0%, 19.7%, and 28.6% higher than the average of the three prior dry seasons at the high-, middle-, and low-elevation sites, respectively.

**Growth and survival of out-plants**

After 41 months, at the end of the fourth dry season post-planting, 53 of the 144 out-plants (36.8%) had died, with 85% of the mortalities occurring during dry seasons. RGR among surviving plants was significantly influenced by source elevation, out-plant site, and the interaction between out-plant site and growth elevation (Appendix S2: Table S3). Plants grown in the upper greenhouse grew at similar rates when planted at the three sites, while growth rates of plants from the lower greenhouse were strongly influenced by the out-planting site: RGR was similar at the low and middle out-planting sites, but was much lower and negative at the high out-planting site (Fig. 7). Source elevation had a weaker effect on RGR, with high-elevation source plants growing significantly more slowly than those from the middle-elevation source location, and low-elevation source plants growing at intermediate rates (Fig. 7). Survival of out-plants was strongly influenced by plant size throughout the entire monitoring period (Table 3), with smaller plants more likely to die than larger plants. When controlling for plant size, low water treatment plants and plants out-planted at their home sites were significantly more likely to survive during the first dry season and first wet season after planting (Table 3, Fig. 8). Inspection of survival patterns at the three sites suggests that this home site effect was driven mainly by lower survival of low- and mid-elevation source plants at the high-elevation site, while source elevation appeared to have little effect at the other two sites (data not shown). Subsequently, water treatment continued to exert a strong effect on survival probability through the second wet season, but began to diminish.
slightly during the third dry season (Table 3, Fig. 8). The influence of home site largely ceased after the first wet season post-planting (Table 3, Fig. 8). Concurrently, out-plant site exerted progressively greater influence on survival probability beginning with the second dry season and continuing through the fourth dry season post-planting (Table 3, Fig. 8). Although patterns shifted somewhat over time, at the end of the fourth dry season, survival was inversely related to the elevation of the three out-planting sites. Source elevation (except as it relates to the home site effect discussed above) and growth elevation appeared to have little effect on survival rates throughout the study period (Table 3). When plant size was not included in models, water treatment and home site were no longer significant influences on survival (Appendix S2: Table S4). Out-plant site was the only significant factor affecting survival rates throughout the study period, with rates consistently being lowest at the high-elevation site, which always had the smallest mean rosette diameter.
DISCUSSION

The response of Haleakalā silverswords to environmental variation appears to be complex. Greenhouse-reared plants exhibited evidence for both heritable variation and plasticity in traits known to influence sensitivity to water stress. Both of these sources of trait variation, likely in combination with differential climatic stress, subsequently contributed to differences in survival of out-plants across the elevational gradient. The complementary greenhouse and common-garden approaches were valuable for evaluating the relative importance of these different mechanisms underlying plant survival. Overall, the findings help explain past patterns of population decline, and in doing so point to potential management strategies for dealing with future drought conditions. More generally, they illustrate how clinal or other geographic variation in phenotypic expression can complicate predictions of vegetation responses to changing climate.

Silversword hydraulic strategy and trait variation

Silverswords appear to possess a hydraulic strategy characterized by isohydry and reliance on a continuous water supply to maintain high levels of photosynthesis and transpiration. Young plants averaged a $\Psi_{soil}$ of $-1.17$ MPa, which is more similar to global means of species from wet tropical rather than dry tropical environments (Bartlett et al. 2012), but is also similar to that of other tropical alpine rosette plants (Rada 2016). This indicates that these plants cannot tolerate very low stem water potentials before loss of leaf turgor and cessation of gas exchange. Transpiration rates of greenhouse plants were quite high, averaging 6.8 mmol H$_2$O m$^{-2}$ s$^{-1}$ among well-watered plants, but similar to those previously measured for wild silverswords (6.6–8.7 mmol H$_2$O m$^{-2}$ s$^{-1}$; Robichaux et al. 1990). Interestingly, Robichaux et al. (1990) found silverswords to transpire at roughly 1.8 times the rate of nearby individuals of the closely related but morphologically divergent shrub Dubautia menziesii (A. Gray) D. Keck. In preliminary, unreplicated field measurements, we similarly found silversword transpiration rates to be 1.7 times those of D. menziesii and nearly 10 times those of the highly sclerophyllous shrub Leptecophylla tameiameiae (Cham. & Schlchtend.) F. v. Muel., in individuals growing within several meters of one another (Appendix S3: Fig. S1).

High water usage by silverswords in their barren, xeric-looking environment appears to be made possible by large investments in their root system. Total root length in 9- to 12-month-old greenhouse plants averaged over 43 m, with some individuals exceeding 100 m. Average root length per leaf area (RLLA, 0.7 m/cm$^2$) in these plants was substantially higher than the modal value measured for mature individuals of a large variety of perennial alpine forbs (Körner and Renhardt 1987). Pérez (2015) found that silverswords preferentially grow on shallow cinder soils <0.4 m in depth at one study site, possibly because of the shorter distance to basalt bedrock that may channel or perch water. We suspect that rooting depth varies across sites according to subsurface geology and pedological properties and, in younger substrates, may correspond to the depth at which porous cinder deposits meet layers that hold or channel more reliable water supplies. Continuity in hydraulic conductance is likely also enhanced by high leaf tissue capacitance, which in silverswords is mediated by longitudinal
channels of pectin-like polysaccharides that bind water, similar to their greensword relative *Argyroxyphium grayanum* (Hillebr.) (Carlquist 1957, Robichaux and Morse 1990). Taken together, the ecohydrologic characteristics of silverswords, like many Andean giant rosette species (Rada 2016), indicate a strategy more aligned with drought avoidance than with drought tolerance (see Appendix S3 for additional information).

In light of this inferred hydraulic strategy, it is perhaps unsurprising that functional trait variation in our greenhouse experiment was strongest among characteristics most likely to influence water uptake and retention. RMF increased in response to the low water treatment and was higher among mid- and high-elevation source plants, while LMF exhibited the reverse pattern. RLSM also increased in response to the low-water treatment, while SLA and LAR decreased when grown in the upper greenhouse. The latter two trait responses may have been driven mainly by lower temperatures that tend to thicken leaves (Körner 1999), but this also has the effect of increasing water-collecting tissue relative to transpiring tissue. Plants grown at high elevation also possessed higher WUE, despite weaker variation in underlying rates of photosynthesis and transpiration. In contrast, we found relatively small, nonsignificant variation in \( \Psi_{tlp} \). This suggests that young silverswords have only limited capacity to lower \( \Psi_{tlp} \), either through osmotic or elastic adjustment, and must maintain relatively high tissue water potentials throughout their range. Although large interspecific differences have been found within related *Dubautia* in both osmotic and elastic properties underlying leaf turgor maintenance (Robichaux et al. 1986), intraspecific plasticity of these traits appears to be more limited (Robichaux 1984).

Seedling trait variation has been tied to differential drought performance, or to ecotypic distributional differences, both among species and among conspecific individuals that experienced contrasting environmental conditions (Zwiazek and Blake 1988, Van den Driessche 1990, Poorter and Marksteijn 2008, Markesteijn and Poorter 2009). The combined trait variation among greenhouse-reared silverswords suggests that, on a size-adjusted basis, plants experiencing lower soil water availability and higher elevation atmospheric conditions during early development, as well as those originating as seeds from higher elevations, should exhibit decreased vulnerability to water shortage. However, all three conditions also reduced growth rates and produced smaller plants. This is potentially important because trait values promoting greater water economy strengthened with increasing plant size, across a wide range of morphological traits. Such size-dependent or ontogenetic trait variation may therefore have implications for the ways in which the underlying causal factors relate to actual drought resistance across the landscape.

### Table 3: Results of GLMs testing the roles of growth elevation, water treatment, source elevation, out-plant site, and home site status on probability of plant survival in out-plant plots.

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<th>Factor</th>
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<th>Wet season 1 (11 months)</th>
<th>Dry season 2 (17 months)</th>
<th>Wet season 2 (23 months)</th>
<th>Dry season 3 (29 months)</th>
<th>Wet season 3 (35 months)</th>
<th>Dry season 4 (41 months)</th>
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<td>0.00 1.000 0.00 1.000</td>
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<td>&lt;0.001 1.000 &lt;0.001 1.000</td>
<td>&lt;0.001 1.000 &lt;0.001 1.000</td>
<td>&lt;0.001 1.000 &lt;0.001 1.000</td>
<td>&lt;0.001 1.000 &lt;0.001 1.000</td>
<td>&lt;0.001 1.000 &lt;0.001 1.000</td>
</tr>
<tr>
<td>Mean diameter</td>
<td>5.51 0.09 5.51 0.09</td>
<td>0.09 0.909 0.09 0.909</td>
<td>0.09 0.909 0.09 0.909</td>
<td>0.09 0.909 0.09 0.909</td>
<td>0.09 0.909 0.09 0.909</td>
<td>0.09 0.909 0.09 0.909</td>
<td>0.09 0.909 0.09 0.909</td>
</tr>
<tr>
<td>Maternal plant</td>
<td>4.78 0.029 4.78 0.029</td>
<td>0.00 1.000 0.00 1.000</td>
<td>0.00 1.000 0.00 1.000</td>
<td>0.00 1.000 0.00 1.000</td>
<td>0.00 1.000 0.00 1.000</td>
<td>0.00 1.000 0.00 1.000</td>
<td>0.00 1.000 0.00 1.000</td>
</tr>
</tbody>
</table>

Notes: Models included average rosette diameter as a size covariate, and maternal plant modeled as a fixed effect. Each model evaluates survival of all out-plants from the time of planting to the end of the specified season (length in months). Significant effects \( (P < 0.05) \) highlighted in boldface type.
**Plant performance under imposed drought and natural field conditions**

Under progressive, terminal drought, all greenhouse plants died at very similar soil water concentrations, indicating that longevity was essentially a function of the rate of water loss through evapotranspiration. The largest determinant of this rate was plant size, with larger plants depleting their water supplies much more quickly. After adjusting for size, plants grown at low elevation perished more quickly when experiencing drought at high elevation, despite the down-regulation of stomatal conductance, which presumably occurred in response to the higher VPD prevailing in the upper greenhouse (e.g., Schulze et al. 1985, Rada 2016). Stomatal control, therefore, appeared to be insufficient to prevent elevated transpirational water loss under the drier atmospheric conditions at higher elevation, which also led to higher rates of soil water evaporation. For reasons that are unclear, a similar pattern did not occur among plants grown at high elevation, which were unaffected by the elevation at which they experienced drought (see also Appendix S3). Overall, the drought experiment suggested that heritable or environmentally induced trait variation had relatively weak effects on plant performance, at least on a size-adjusted basis under greenhouse settings. Adjusting for size is likely to be especially important when evaluating performance of

**FIG. 8.** Fitted survival curves of the out-plants over time. Each column of plots shows the effects of the factor indicated at the top of the column, adjusting for all other factors in the models, and plotted against plant size. Each row of plots shows temporal changes in the effects, from the time of out-planting through the end of each dry season indicated. There were no qualitative changes in the patterns during the intervening wet seasons (see Table 3). Statistical significance of effects is indicated next to the temporal label in each plot: *P < 0.05, **P < 0.01.
plants confined to relatively small pots, because the use of pots unrealistically eliminates differences in access to soil water that would probably occur among differently sized plants in the field via unequal rooting depths and volumes. The application of a progressive drought on potted plants may have also imposed unrealistically severe conditions, and could have masked subtler influences of trait variation if water deficits typically develop more slowly or intermittently in the field.

The common-garden out-planting component circumvented these limitations, but at the cost of uncontrolled precipitation and soil moisture conditions. Because unusually wet weather prevailed for much of the first 2–3 yr after out-planting, a fairly long monitoring period was needed to capture meaningful variation in environmental conditions and to observe survival patterns during a more stressful dry season. This experiment indicated that the watering treatment prior to out-planting had a significant influence on plant survival that persisted until the end of the third dry season after planting. Low water treatment plants survived at higher rates than high water treatment plants, on a size-adjusted basis, presumably because the greater relative investments in below-ground tissues resulted in more effective water management (Lloret et al. 1999). In contrast, the growth elevation prior to planting did not have a significant effect on out-plant survival, despite also inducing plastic trait responses. It did, however, influence growth rates after out-planting. Plants grown in the lower greenhouse increased in rosette diameter when planted at the low and mid-elevation sites, but decreased when planted at the high-elevation site. There was also a significant home site advantage over the first 11 months after out-planting. This evidence for local genetic adaptation, however, was mainly limited to better survival of high-source-elevation plants at the high out-planting site, whereas source elevation had little influence at the two lower sites.

Both the water treatment and home site effect diminished over time, while the influence of out-plant site increased over time, becoming statistically significant during the final 18 months of the study. Higher survival was positively associated with site elevation at the end of the study, which matches out-plant survival patterns in a pilot effort conducted at the same three sites (P. Krushelnycky, unpublished data), and is consistent with the elevational pattern of mortality observed in the wild population over the previous few decades (Krushelnycky et al. 2013, 2016). An attenuating influence of treatment effects that arose prior to out-planting makes sense, as trait plasticity can be expected to increasingly respond over time to the local conditions at the out-plant locations. However, this also makes it more difficult, as time progresses, to distinguish between the effects of strengthening inter-site trait differences and the effects of differential climatic stress in driving the contrasting levels of mortality at the three sites.

Climate was clearly influenced by ENSO conditions during the first few years of the study period, resulting in higher than normal rainfall and lower than normal VPD during the first three dry seasons after out-planting. Soil moisture should provide an integrated measure of plant water availability resulting from differences in precipitation and evaporative demand, and was unusually constant at each site during the first three years after out-planting owing to the elevated dry season rainfall. Unfortunately, differences in upper soil substrates made among-site comparisons of soil moisture measurements invalid. An assessment of relative climatic stress was further complicated by the fact that plant mortality was not correlated with the same climate variables at the three sites. In general, it was surprisingly difficult to identify consistently strong climatic drivers of out-plant mortality, and to disentangle the influences of inherent plant differences from external forcing conditions. Both factors may have played a role: silverswords planted at lower elevations likely became increasingly less drought-resistant relative to those planted at higher elevations,

![Fig. 9. Climate variables most strongly related to probability of out-plant mortality during the previous monitoring quarter at the three out-plant sites. At high elevation, interacting effects of low and high values (25th and 75th percentiles) of soil moisture (SM, black curves) and vapor pressure deficit (VPD, red curves) with air temperature (°C) are depicted (high VPD curve is coincident with low SM curve). VPD (hPa) and SM (m³/m³) were most strongly related to probability of mortality at the mid- and low-elevation sites, respectively. All probabilities calculated at mean rosette diameter and at mean values of other climate variables included in composite averaged models for each site (Appendix S2: Table S5).](image)
but also experienced larger relative reductions in soil moisture and larger relative increases in VPD during the final harsh dry season.

Because size was an important determinant of survival probability throughout the out-planting study, removing the size adjustment from analyses shifted survival rates in favor of groups with larger mean rosette sizes. This resulted in high water treatment plants no longer being more likely to die than low water treatment plants, and in a smaller home site advantage that was only marginally statistically significant. Survival rates were lowest at the high-elevation site, initially because out-plants were smaller at that site by chance, and subsequently because slower growth rates at high elevation enhanced this size difference. The ways in which the factors discussed above influence drought resistance and survival across the silversword range may therefore vary temporally. In recent decades, silversword demographics have been characterized by infrequent bouts of successful recruitment (Krushelnycky et al. 2013). Immediately after such recruitment events, factors that promote faster growth (greater water availability, lower elevation atmospheric conditions) may be advantageous if they allow seedlings to escape vulnerable sizes more quickly. Outside of these periods, however, factors that enhance drought resistance on a size-adjusted basis (lower water availability, higher elevation conditions), that act on broader populations possessing a wide range of age and size classes, likely become more important.

Conservation insights and broader implications

The considerable plasticity exhibited by silverswords has the potential to buffer them against future climate changes (Valladares et al. 2014). However, this may depend strongly on the pace and nature of such changes. Relatively gradual declines in precipitation could induce adjustments that strengthen drought resistance and minimize or forestall increases in mortality. In contrast, more rapid reductions in rainfall, or changes that are characterized by intensifying extremes within relatively similar background conditions, could promote higher mortality in areas that, on average, experience more benign conditions and induce less resistant phenotypes (e.g., Ogle et al. 2000). The balance of information supports the latter scenario in recent decades, and suggests that the enhanced mortality in the lower portions of the silversword range has been driven principally by the acquisition of trait characteristics that reduce drought resistance of plants growing in these regions, which then suffer higher rates of mortality during intensified dry seasons. This effect may be strengthened by larger departures from typical conditions in lower elevation regions, which more frequently experience cloud inundation and humid air owing to their closer proximity to the base of the TWI. If future climate changes are characterized by intensifying variability or extremes more so than by changing averages (McLaughlin et al. 2002, Jentsch et al. 2007, Feng et al. 2013), plants growing in relatively wetter portions of the current range may continue to experience higher relative rates of mortality. The resulting spatial pattern, greater retraction from wetter rather than drier portions of the distribution, would essentially be opposite to that predicted under a drying scenario that assumes no intraspecific variation in drought resistance (i.e., a single, broad moisture tolerance range).

Silverswords also exhibited heritable trait differences that should enhance drought resistance in high-elevation populations relative to lower elevation populations, but these did not appear to have a strong influence on survival patterns of out-plants. Genetic differentiation may have been overshadowed by the effects of plasticity, as suggested by the relatively short-lived home site advantage at the high-elevation out-planting site. The absence of more consistent survival advantages of high-elevation source plants implies that genetic mixing is not likely to be a highly effective strategy for enhancing drought resistance across the wider population. Identification of the most favorable habitat locations for current and future propagation may therefore be the most effective conservation strategy going forward (e.g., Marrero-Gómez et al. 2007). Silverswords are known to have previously occupied wetter areas outside the current range, and although plants propagated in these wetter regions can be expected to develop phenotypes that are less drought resistant, future precipitation in those regions may still be high enough, even under drying scenarios, to allow population persistence. Our study also suggests that more restrictive watering regimes may enhance silversword out-plant survival during the early periods after establishment. The benefits of larger plant size can be attained under lower watering regimes by simply utilizing a longer greenhouse growth period.

More broadly, it will be valuable to understand whether the inferred isohydric, drought-avoidance strategy of silverswords and their apparent reliance on high soil water availability has made them more or less vulnerable to changing conditions than either co-occurring species or those growing elsewhere. West et al. (2012) linked contrasting hydraulic strategies to differential drought sensitivity in another Mediterranean-type ecosystem, but found deep-rooted isohydric species to be less vulnerable than more shallow-rooted and anisohydric species. Whether this pattern differs from plant communities at Haleakalā is currently difficult to assess because of the absence of comparable data for sympatric species, but this question represents a profitable future line of inquiry. Additional ecophysiological studies, especially comparative ones, will therefore help determine the generality of such interspecific patterns. Even single-species case studies can strengthen modeling forecasts by providing insight into how intraspecific variation in climatic tolerance may produce different patterns from those predicted when assuming a single climatic envelope. In this respect, our results support a growing body of findings indicating that the consideration of clinal or
other geographic variation is likely to produce more accurate assessments of a species’ future range shifts and vulnerability (e.g., O’Neill et al. 2008, Kuo and Sanford 2009, Valladares et al. 2014, Ikeda et al. 2017).

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Literature Cited


**Supporting Information**

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecm.1398/full

**Data Availability**

Data are achieved at ScienceBase: https://www.sciencebase.gov/catalog/item/57dc6668e4b090824ffdb9ea.