

LOCAL ADAPTATION OF THE HAWAIIAN ENDEMICE TREE (*Metrosideros polymorpha*)
ACROSS A LONG ELEVATION GRADIENT

by

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A Thesis Submitted in
Partial Fulfillment of the
Requirements for the Degree of

Masters of Science

in Tropical Conservation Biology and Environmental Science

at University of Hawai'i at Hilo

July 2015

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Acknowledgements

I am using this opportunity to express my gratitude to everyone who supported me throughout the course of my thesis project. In particular, I would like to express my special appreciation and thanks to my advisor Dr. Elizabeth Stacy for being a tremendous mentor for me. I would like to thank you for your support to grow as a researcher as well as your advice on both research and my career. I would also like to thank my committee members, Dr. Abby Cuttriss and Dr. Donald Price for providing me with professional guidance and sharing illuminating views on a number of issues related to the project. Thank you to Dr. Patrick Hart, Dr. William Sakai, Dr. Rebecca Ostertag, Dr. Yohan Pillon, and Dr. Yiqing Li for helpful discussions. I express warm thanks to Jennifer Johansen, Janis Iyo, Jill Ekar, Lynx Gallagher, Ala Malia Leka, Erin Datlof, Linnea Heu, Corie Yanger, Christy Short, Naomi Hagura, Eric Taylor, Merritt Burch, Matt Lodge, Matt Force, Jodie Rosam, Eva Brill, Cade Howe, and Sherry Okuyama for project assistance and friendly advice. In addition, a thank you to Tim Ambrose, Kim Snuggerud, and Mike Mathews for allowing me to set a HOBO Weather Station in your property. I also would like to thank to the National Science Foundation CREST Award for the financial support. Without their support, this project was not accomplished. Lastly, I recognize that this research would not have been possible without the resources shared by UH Hilo Natural Science Division, UH Hilo CAFNRM, UH Hilo Marine Science Department, Hawai'i Island DOFAW, and HAVO, and express my gratitude to those institutes and agencies.

Abstract

Elevation gradients are important drivers of divergence in trees, yet little is known about the spatial scales over which divergence occurs, nor the abiotic factors that drive divergence. The endemic Hawaiian tree, 'ōhi'a lehua (*Metrosideros polymorpha*) spans a striking elevation gradient on Hawai'i Island, from near sea level to 2,470 m, and comprises two pubescent varieties; *M. polymorpha* var. *incana* tends to be found at lower elevations, *M. polymorpha* var. *polymorpha* is limited to higher elevations, and purported hybrids occur at middle elevations. To better understand the scale and drivers of divergence in tree populations along elevation gradients, I conducted field, greenhouse, and growth-chamber experiments on open-pollinated seedlings from six populations of pubescent *M. polymorpha* spanning the full elevation range of this species on Hawai'i Island. The reciprocal outplanting experiment revealed universally low survivorship at the extreme ends of the elevation gradient, suggesting these environments are the most challenging. Results of the 12-month UV-light experiment in the greenhouse revealed relatively lower survivorship under high-UV light of seedlings from the three highest-elevation populations (counter to expectations), contrasting relationships between anthocyanin concentration and survivorship between the two varieties, and possible heat sensitivity of high-elevation seedlings. Lastly, short-term stress response tests were done under extreme environmental conditions (i.e., high UV radiation and low temperature) in a growth chamber. No seedlings from the highest-elevation population died after exposure to -5°C for four nights, while seedlings from all other populations had lower survivorship. These results indicate local adaptation at both the variety level and suggest that both UV radiation and temperature are important drivers of local adaptation of trees across long elevation gradients.

Introduction

Local adaptation

Local adaptation is an important process in evolution that causes organisms to be differentiated among habitats through divergent selection (Rundle & Nosil 2005). Environmental variation creates selective pressures on individuals favoring certain traits in populations in a particular environment that promote high fitness (Rundle & Nosil 2005). Because a locally adapted population performs better in its home range (Leimu & Fischer 2008), the traits that increase individual fitness are expected to persist or increase in frequency in a population from one generation to the next. Within widespread species, local adaptation results in genetic divergence between populations found in different environments, which may eventually lead to the accumulation of reproductive isolating barriers and speciation (Rundle & Nosil 2005; Savolainen et al. 2007). For example, restricted gene flow was demonstrated between two subspecies of the herbaceous *Gilia capitata* locally adapted to different habitats (Nagy and Rice 1997). To what extent populations specialize to their local environment is a key question in evolutionary biology, because it determines, for example, the potential for speciation (Gavrilets, 2003; Blanquart et al. 2012)

Two criteria must be met for populations to be locally adapted to different environments according to Kawecki and Ebert (2004). The first criterion focuses on differences in fitness between local and foreign populations in habitats: local populations must have a higher fitness than foreign populations in their local conditions. The second criterion concerns fitness differences between populations inside and outside of their home ranges: a population must have higher fitness in its home habitat than in foreign habitats. Local adaptation therefore occurs when the fitness of each population is relatively higher in its home habitat (Kawecki & Ebert 2004; Savolainen et al. 2007). However, the combination of these two criteria may be unnecessarily strict. When two populations are compared as a simple example, both populations could have absolute higher or lower fitness at one of the habitats, and relatively higher fitness in their home environments. In such a case, the first criterion is satisfied but the second is not. Such a scenario would be expected, for example, when one habitat is very challenging and the fitness of both populations is low. We could also imagine another situation in which the first criterion is not satisfied but the second criterion is; one population performs better in both habitats, but fitness of that population is lower in the foreign habitat than at home. The third possible situation could be when one population shows evidence of local adaptation but the other does not. To overcome the effects of varying habitat and population qualities, a new approach was suggested to estimate local adaptation based on the mean fitness of all populations of interest by improving the idea of two criteria proposed by Kawecki and Ebert (2004) (reviewed in Blanquart et al. 2013). Using this approach, local adaptation would be detected in all three scenarios above. This approach improves statistical power as well as estimation of local adaptation by emphasizing which genotypic composition is more suitable to an environment without ignoring evidence of local adaptation due to poor habitat/population quality (Blanquart et al. 2013).

The evolutionary mechanisms that are most important for local adaptation are gene flow and selection (Garcia-Romas & Kirkpatrick 1997; Kawecki & Ebert 2004; Savolainen et al. 2007; Blanquart et al. 2013). Restricted gene flow and strong selection allow populations to diverge, whereas extensive gene flow and weak selection prevent populations from differentiating (Garcia-Romas & Kirkpatrick 1997). In tree species for which seed and pollen movement is restricted to short distances, isolation of populations by distance alone should occur due to the lack of gene

flow ($m \approx 0$) (Garcia-Romas & Kirkpatrick 1997). Even if seeds and pollen are dispersed into a new habitat ($m > 0$), immigrants may experience strong selective pressure under new conditions, which may inhibit establishment in the area (Charlesworth et al. 1982). If dispersal distance is short and/or selection is strong over short distances, local adaptation can be detected over a fine spatial scale (Savolainen et al. 2007). Even where selection is weak, short dispersal distance can lead to isolation by distance, or the divergence of populations due to genetic drift alone (Savolainen et al. 2007; Blanquart et al. 2013). On the other hand, high gene flow between populations ($m \approx 1$) prevents populations from adapting to local environmental conditions by mixing alleles (Garcia-Romas & Kirkpatrick 1997). Where gene flow occurs at intermediate levels, divergent natural selection must be strong enough to overcome the homogenizing effects of gene flow (Savolainen et al. 2007).

Plant adaptation along elevation gradients

Elevation gradients can drive divergence in plant populations. A meta-analysis of local adaptation in plants and animals indicated a significant positive relationship between local adaptation and environmental variables such as elevation (Hereford 2009). Although local adaptation along elevation gradients is not well studied, some studies support the existence of ecologically diverged plant species, subspecies, and populations across elevations, suggesting a role for elevation in divergence (Galen et al. 1991; Rice & Mack 1991; Wang et al. 1997; Flegrova & Krahulec 1999; Link et al. 2003; Angert & Schemske 2005; Gimenez-Benavides et al. 2007). For example, two closely related species, diploid *Anthozanthum alpinum* and tetraploid *A. odoratum* were believed to have distinct distributions with a boundary at 1000 m above sea level (a.s.l.) in the Krkonoše Mountains, and were reciprocally transplanted between 880 m and 1100 m (Flegrova & Krahulec 1999). Individuals of the arctic-alpine species *A. alpinum* planted at 1100 m had higher survivorship and increased tussock growth compared to those at 880 m, while the lower elevation species *A. odoratum* had higher survivorship and flowering rates at 880 m. The reciprocal transplant experiment suggested that both species favor their local environments. A more recent detailed mapping study revealed overlapping distributions of the two species from 800-1290 m a.s.l. (Filipova & Krahulec 2006). Thus, these two species show clear evidence of differential local adaptation to higher and lower elevations despite their highly overlapping ranges. A common garden experiment of Norway spruce (*Picea abies*) collected across elevations and planted at high elevation also suggested that selection may cause high- and low-elevation trees to become differentiated (Oleksyn et al. 1998). For some species, hybridization occurs naturally where elevation ranges overlap, and hybrids show evidence of local adaptation in these regions. Between two big sagebrush subspecies (*Artemisia tridentata* ssp. *tridentata* and *vaseyana*) which are parapatrically distributed, hybrids occur in the middle elevations (Wang et al. 1997). Highest fitness was observed in hybrids grown within hybrid zones as well as in parental taxa within their native habitats. Although hybrids have genes adapted to different parental environments, the combination of genes found in hybrids can be favored in hybrid zones (Wang et al. 1997). Such a pattern implies that stabilizing selection acts on hybrids due to genotype and environment interactions (Wang et al. 1997). According to a reciprocal transplanting experiment in *Chamaecrista fasciculata* along a longitudinal gradient, the most extreme ecological distance (differential local adaptation) was found between populations occupying the extreme ends of the gradient, while there was no linear relationship between ecological difference and geographical distance (Galloway & Fenster 2000). Although their experiment did not support the hypothesis of

isolation-by-distance, it suggests the presence of environmental differences that create divergent selection pressures at the two ends of the gradient.

The magnitude of local adaptation can differ between plant taxa along elevation gradients. According to an experiment of transplanted seeds within and outside the natural ranges of two sister monkey flower species, the higher elevation species (*Mimulus lewisii*) may be more restricted to high elevations, while the lower elevation species (*M. cardinalis*) has the potential to survive even beyond the upper limit of its current elevation range (Angert & Schemske 2005). These two species occur in different elevations with sympatric regions at intermediate elevations. The seeds used for the experiment were produced by outcrossing among populations within species, thus representing a breadth of genetic variation within each species. In contrast to these results, another reciprocal transplant experiment at the population level illustrated an opposite pattern. The lower elevation population of a perennial bunchgrass (*Poa secunda*) was more sensitive to high-elevation conditions, whereas growth of plants from a higher elevation population was not reduced by the environmental conditions at lower elevation (Link et al. 2003). Thus, the strength of local adaptation of plant species does not appear to vary predictably across elevation gradients.

Gene flow is one of the key factors that determines the degree of local adaptation along elevation gradients as described above. However, extensive gene flow may not necessarily hinder local adaptation. For example, a perennial grass (*Festuca eskia*) exhibited low genetic diversity (presumably high gene flow) along three elevation transects from 1500 to 2500 m, and high genetic diversity (presumably low gene flow) along a longitudinal gradient on the Pyrenean Mountains. Plant survivorship from a reciprocal transplant experiment revealed that populations at high elevation were locally adapted, and divergence of adaptive traits was detected over a short distance between low and high elevations (Gonzalo-Turpin & Hazard 2009). This result suggests that divergent selection may be sufficiently strong to overcome the homogenizing effects of gene flow. Whereas such a result might be expected for trees for which extensive gene flow among populations is common (Petit & Hampe 2006), this experiment demonstrates that a grass species also has the ability to adapt to local conditions when gene flow is high. A continuous distribution along elevation gradients allows plants to exchange pollen and seeds over long distances directly (Dick et al. 2008) or by using intermediate elevations as stepping stones (Schuster et al. 1989). Therefore, a high level of gene exchange along elevation gradients may be a natural feature in plants (Petit & Hampe 2006; Gonzalo-Turpin & Hazard 2009). As such, whereas elevation may provide plants with a range of selective pressures, it likely does not lead to the genetic isolation of plant populations through reduced gene flow only (Navascues et al. 2008). Therefore, habitat heterogeneity should be an important driver of differentiation along elevation gradients rather than geographical distance (Leimu & Fischer 2008).

Drivers of local adaptation along altitudinal gradients

While local adaptation in plant populations along altitudinal gradients has been documented, understanding the drivers of local adaptation along such gradients is still limited. Significant variation in abiotic factors such as temperature, UV radiation, and water availability are reported across elevation gradients (Loope & Giambelluca 1998; Link et al. 2003; Gimenez-Benavides et al. 2007; Terfa et al. 2014), each of which is expected to influence local plant populations (Fryer & Ledig 1972; Korner & Woodward 1987; Sullivan et al. 1992; Ziska et al. 1992; Angert & Schemske 2005; Gimenez-Benavides et al. 2007).

Temperature is often cited as a factor that limits plant distributions, although the degree of sensitivity to temperature change varies significantly among populations (Link et al. 2003; Gimenez-Benavides et al. 2007) and species (Angert & Schemske 2005). Higher temperature with lower soil water content may inhibit successful seedling establishment of higher elevation plants of *Silene ciliata* at lower elevations (Gimenez-Benavides et al. 2007). The higher elevation species *Mimulus lewisii* may be more sensitive to low-elevation environments than the lower species *M. cardinalis* to high-elevation conditions (Angert & Schemske 2005). Although the effect of high temperature was not directly tested on high-elevation species in their study, heat sensitivity of *M. lewisii* was suggested by unpublished data from growth chamber experiments (Angert & Schemske 2005). In contrast, the lower temperatures and wetter conditions at a higher elevation (844 m) reduced plant growth for lower elevation populations of *Poa secunda* (Link et al. 2003). Leaf expansion in grass (*Poa*) species corresponds to temperature; high-elevation plants were capable of leaf expansion below the freezing point, whereas leaf expansion of a low-elevation population was faster at higher temperatures (Korner & Woodward 1987). Photosynthetic CO₂ uptake was a physiological adaptation to change in temperatures along altitudinal gradients reported in balsam fir (*Abies balsamea*) (Fryer & Ledig 1972). Temperature optima for photosynthetic CO₂ uptake decreased with increased elevations. Slow growth rates of trees at high elevation have been attributed to low temperature (Oleksyn et al. 1998).

Solar UV radiation and elevation are only weakly correlated because the former is strongly affected by cloud cover (Blumthaler et al. 1997; Korner 2007); however, UV radiation generally increases within elevation (Sullivan et al. 1992; Terfa et al. 2014). An effect of elevation on UV irradiation was suggested, and an increase in UV irradiation by about 8% per 1000 m was observed according to broad measurements at Jungfrauoch, Switzerland and Innsbruck, Austria (Blumthaler et al. 1997). In addition, a linear relationship between solar radiation and elevation was reported above the inversion layer at 1650 m on Maui, Hawaii (Loope & Giambelluca 1998). Ultraviolet radiation is categorized into three types: UV-A (320 – 400 nm), which is ~6 % of incoming solar radiation and less harmful to plants; UV-B (280 – 320 nm), which comprises only ~1.5 % of incoming solar radiation but damages DNA; and UV-C (200 – 280 nm), which is mostly filtered out by a stratospheric ozone layer but which can have severely damaging effects on plants (Levitt 1972; Hollosy 2002). High UV radiation can damage plants and affect growth and development (Robberecht et al. 1980), including shoot length, leaf area, and leaf thickness (commonly induced by UV-B; Jansen et al. 1998). Although strong effects of UV radiation on plants are often described, only a few direct tests of adaptation to UV radiation have been performed. Reduced sensitivity of high-elevation populations to UV-B radiation has been illustrated in Hawaiian plants (Sullivan et al. 1992; Ziska et al. 1992). While the responses to UV-B radiation of both endemic and introduced species in Hawai'i varied among plants from different elevations, an increase in biomass under high UV-B was detected in plants from higher elevations relative to those from lower elevations (Sullivan et al. 1992). A persistently greater concentration of UV-B-absorbing pigments (e.g. flavonoids) was found in the high-elevation plants regardless of UV-B radiation exposure, while increased flavonoid content was demonstrated in low-elevation populations only under increased UV-B radiation. These results suggest that flavonoid synthesis is a fixed adaptation of high-elevation plants that facilitates productivity under the stress of high UV-B radiation (Ziska et al. 1992). Anthocyanins are a class of pigments that are also responsible for protecting plants against damage induced by environmental factors, including UV radiation (Levitt 1972; Chalker-Schott 1999; Gitelson et al. 2001). Increase in concentration of anthocyanins with elevated UV levels is documented in lettuce (*Lactuca sativa*; Paul et al. 2012).

*Local adaptation in *Metrosideros polymorpha* in Hawai‘i*

Native forests on the Hawaiian Islands are dominated by the endemic woody genus *Metrosideros*. *Metrosideros* first colonized the Hawaiian Islands approximately 4 million years ago from the south Pacific (Percy et al. 2008). One of five Hawaiian *Metrosideros* species, *M. polymorpha*, known locally as ‘ōhi‘a lehua, is a notably dominant species on the Hawaiian Islands (Dawson & Stemmermann 1990; Percy et al. 2008). This single species is highly polymorphic, living in a wide range of habitats that vary in substrate age, elevation, temperature and precipitation (Corn & Hiesey 1973; Mueller-Dombois 1980; Cordell et al. 1998). There are eight described varieties within *Metrosideros polymorpha* that are distinguished by vegetative characters (Dawson & Stemmermann 1990; Friday and Herbert 2006), though many other forms can be recognized in the field (J. Lau and E. Stacy, pers. obs.).

The Hawaiian Islands provide an ideal setting for the study of ecological and evolutionary processes. One of the defining characteristics of the Hawaiian Islands is that abiotic factors such as temperature, precipitation and nutrient availability change markedly over short distances; this is especially true along altitudinal gradients. These conditions allow us to study how environmental factors affect the dominant plant species, *Metrosideros polymorpha*. On Hawai‘i Island, *M. polymorpha* dominates the native landscape from sea level to ~2,470 m above sea level wherever annual rainfall exceeds 500 mm (Stemmermann & Ihsle 1993), and comprises four partially but significantly isolated varieties (Stacy et al. 2014). A study of leaf structure and pigment concentration in a common garden illustrated that leaves of *M. polymorpha* collected at high elevations on Mauna Loa were thicker and denser and had more leaf pigments than leaves from low elevations (Martin et al. 2007). The two high-elevation populations (1,980 m and 2,470 m) used in this study correspond to the high-elevation var. *polymorpha*, while pubescent trees at low elevation correspond to the early-successional var. *incana*. These results suggest that there might be interesting differences in light absorption between varieties *incana* and *polymorpha*. Abiotic factors that may drive local adaptation within *M. polymorpha* along an elevation gradient at the scale of either populations or varieties are UV radiation, temperature, water availability, and nutrient availability. None has been examined yet to my knowledge.

Metrosideros polymorpha var. *incana* and var. *polymorpha*

Pubescent-leaved varieties, *M. polymorpha* var. *incana* and var. *polymorpha*, are typically found on young lava flows, although var. *polymorpha* can be found on old substrate (Stemmermann 1983). Early-successional var. *incana* has small leaves covered with short pubescence on the lower surface and occurs on dry sites at low to middle elevation on O‘ahu, Moloka‘i, Lana‘i, Maui and Hawai‘i (Dawson & Stemmermann 1990). Relative to the glabrous, late-successional var. *glaberrima* that dominates wet forests at low to middle elevations, var. *incana* is significantly more light demanding at the seedling stage (Morrison & Stacy 2014). In contrast, the range of var. *polymorpha* is from middle to higher elevations on Moloka‘i, Maui and Hawai‘i (E. Stacy and J. Johansen pers. obs.). Variety *polymorpha* has small and thick leaves with dense woolly pubescence on the lower surface (Dawson & Stemmermann 1990; Friday and Herbert 2006), and the petiole and internode lengths are shorter than those of var. *incana* (Cordell et al. 1998). Another phenotypic difference between these varieties is style length, which is significantly greater in var. *polymorpha* (Johansen & Stacy, unpub. data). Divergent style lengths may reflect adaptation to different pollinator communities and may account for the reduced pollen tube length and seed germination observed in crosses between var. *incana* (pollen donors) and var.

polymorpha (maternal trees) compared to within-variety crosses (Rhoades 2012). On Mauna Loa, Hawai'i, var. *incana* is distributed from sea level to 900 m, and var. *polymorpha* from 1800-2400 m (based on morphology; Johansen. pers. obs.). At intermediate elevations, purported hybrids among the two varieties as well as glabrous variety, var. *glaberrima*, are common.

M. polymorpha vars. *incana* and *polymorpha* are ideal varieties for the study of local adaptation not only because their population sizes are sufficiently large to minimize the impact of genetic drift on genetic differentiation and local adaptation (Savolainen et al. 2007), but also because together they span a steep elevation gradient on Hawai'i Island along which environmental conditions are expected to vary dramatically. The continuous distribution of these pubescent varieties allows study of the effects of environmental conditions on local adaptation and divergence within a tree species. *M. polymorpha* vars. *incana* and *polymorpha* on Hawai'i Island are partially though significantly isolated at neutral genetic loci (Stacy et al. 2014). Genetic distance between these varieties estimated for populations occurring on Mauna Loa was low but significant (mean pairwise $F_{ST} = 0.046$; Stacy et al. 2014). According to Schuster's et al. (1989) method of investigating the effect of drift on genetic differentiation between populations, there are approximately 5.18 migrants exchanged between the two varieties per generation, indicating the effect of drift is negligible since there are more than one migrants per generation. A larger genetic distance was found between vars. *incana* and *polymorpha* on Mauna Loa ($F_{ST} = 0.105$; DeBoer & Stacy 2013). Even in this case, genetic drift would still be too weak to lead to genetic differentiation between the varieties (2.13 migrants per generation).

The objectives of this study were to determine if populations of the landscape-dominant tree, *M. polymorpha*, are adapted to local environments along a striking elevation gradient on Hawai'i Island and to understand how important UV radiation and temperature are to local adaptation across elevations. The study focuses on purely pubescent populations comprising vars. *incana* and *polymorpha* to avoid the influence of the late-successional, wet-forest var. *glaberrima*, which is known to differ from sympatric var. *incana* in light requirements at the seedling stage (Morrison & Stacy 2014). Given that both of the targeted varieties occur also on older islands within the archipelago (var. *polymorpha* on Maui nui, and var. *incana* on all islands except Kaua'i) and that they likely colonized young Hawai'i Island independently (Stacy et al. 2014), Hawai'i Island likely represents the conditions in which the two varieties originated on an older island when it was still volcanically active. Study results therefore will yield insight into how elevation gradients drive adaptive divergence within long-lived tree species as well as important ecological differences between two of the three dominant forms of *M. polymorpha* on Hawai'i Island.

Method

Seed collection sites and germination

Six sites were selected for seed collection of pubescent *M. polymorpha* spanning the full elevation range of this species (i.e., from near sea level to 2300 m above sea level) on East Hawai'i Island (Fig. 1). All sampled populations comprised 90-95% pubescent trees of *M. polymorpha*; populations containing apparent hybrids with the glabrous variety *M. polymorpha* var. *glaberrima* were avoided. Trees were identified as a pubescent type by observing pubescent characteristics. The two pubescent taxa (*M. polymorpha* vars. *incana* and *polymorpha*) were distinguished based on morphological characters (Table 1).

In 2012, open-pollinated seeds were collected from 5-10 fruit capsules from each of approximately 25 trees at each of the six study sites, and were stored in coin envelopes in a dry room for 1-2 months before sowing. Seeds from three capsules from each maternal tree were sown across two wells in a Superior Growers 1204 series seed-starting tray in the UH Hilo Pana'ewa Farm greenhouse in 2013. Trays were misted every 30 min for 20 sec (day time only) under low light intensity (approximately 600 $\mu\text{mol}/\text{m}^2/\text{s}$) and almost zero UV light intensity. Visible and UV light intensity were measured using a photosynthetically active radiation (PAR) meter and ultraviolet meter, respectively (Apogee Instruments, Inc., Logan, UT, USA). Germination occurred on low-nutrient medium (Sunshine Mix #4). Two months after germination, seedlings were moved to another greenhouse and watered three times a day for five minutes under higher photosynthetic and UV light intensities (approximately 700 $\mu\text{mol}/\text{m}^2/\text{s}$ and 35 $\mu\text{mol}/\text{m}^2/\text{s}$, respectively).

Reciprocal transplant experiment

To estimate the strength of local adaptation across the elevation gradient, six seedlings were chosen by size from each half-sib family (1 seedling/family \times 6 out-planting sites \times 20 families), and each seedling was transplanted into a small well with the same medium used for germination six months after seedlings were transferred to the second greenhouse. If a seedling died within a week due to transplant shock, it was replaced with a new seedling from the same family.

Initial sizes of all seedlings were recorded in terms of the number of stems, main stem length (cm), cumulative stem length (cm), the number of leaves, leaf length (mm) and leaf width (mm) from the largest leaf, root length (cm), and presence of petioles and pubescence. Seedlings were transported after all soil was thoroughly washed away from the roots. Roots were wrapped in sterile sphagnum moss and paper towels that were soaked in water to prevent spreading soil-borne pests.

Seedlings were randomly selected from 20 families for each of the six source populations to out-plant in seven 2' \times 2' plots at each of the six outplanting sites (3 families/population \times 6 source populations in 6 plots; 2 families/population \times 6 source populations in 1 plot). At a single site, seven plots were situated discreetly (20 families \times 6 source populations = 120 seedlings/site). Any seedling that died within one month was replaced with another seedling from the same family. Since approximately 75% of the seedlings at the 90-m site died in less than one month probably due to drought conditions, trampoline-style shade cloth was set over surviving seedlings and the replaced seedlings after planting during the experiment. Seedlings were monitored monthly for survival and any damage for 12 months after outplanting. At the end of the experiment, all

measurements taken prior to outplanting, except root length, were recorded. Since outplanting of seedlings was not permitted at the Ka'u Desert and Mauna Loa Strip Road sites managed by Hawai'i Volcanoes National Park, analogous outplanting sites were selected on state forest land at the same elevations (Fig. 2).

To allow between-site comparisons of annual variation in environmental conditions, a HOBO U30-NRC Weather Station (Onset Computer Corporation, Bourne, MA, USA) was placed at each of the six outplanting sites to record relative humidity (RH), PAR, and temperature for one year.

UV light experiment in the greenhouse

Three UV light levels were set up in a greenhouse covered with the ethylene-tetrafluoroethylene copolymer (ETFE) film, where a large percentage (e.g. ~88%; 166.8 $\mu\text{mol}/\text{m}^2/\text{s}$ outside the greenhouse, 146.7 $\mu\text{mol}/\text{m}^2/\text{s}$ under the ETFE film in June 2013) of the ultraviolet light was transmitted (Sakai et al. unpub.; Shiohita et al. unpub.). Ambient UV light intensity through the ETFE film was used as the low-UV light treatment for the experiment, which was nearly identical to the UV intensity at the lowest-elevation seed collection site (~200 $\mu\text{mol}/\text{m}^2/\text{s}$). The intensity of visible light was ~2000 $\mu\text{mol}/\text{m}^2/\text{s}$ under the ETFE film on a cloudless day. To create a high-UV light condition, UV transmission was supplemented by UV fluorescent lamps (Zoo Med Reptisun T5 HO 10.0 UVB High Output Bulbs, PetMountain, CA, USA), which produced ~130 $\mu\text{mol}/\text{m}^2/\text{s}$, in addition to ambient UV radiation through the ETFE film. As a control (i.e. UV light < 1 $\mu\text{mol}/\text{m}^2/\text{s}$), UV light was blocked by a polycarbonate-acrylic (PC-A) panel. The maximum and minimum temperatures were recorded under each of the three UV light levels every two weeks.

For each treatment, 40 approximately 1-cm-tall seedlings from each collection site were randomly chosen (2 seedlings/family x 20 families), three months after the seedlings were transferred to the second greenhouse. Each seedling was transplanted into a 2.5-cm-diameter dibble tube with a nitrogen-free medium (Sunshine Mix #4). If any seedling died within a week, a new seedling was used from the same family. At the time of transplantation, the following measurements were recorded: shoot length (mm), the number of leaves, the number of nodes, leaf width (mm), and leaf length (mm). The largest leaf was selected to measure leaf width and length. After seedlings were established in dibble tubes for two months, seedlings were placed under the three UV light settings (40 seedlings/collection site x 6 collection sites = 240 seedlings/treatment). The seedlings were rotated semimonthly within a treatment to minimize location effects. Watering was done via a tray with small holes placed under the dibble tubes and filled with water and slowly drained each day. To examine how seedlings were influenced by UV light over time, survivorship, the presence of pubescence, and herbivore damage on seedlings were observed every two weeks. After one year of exposure, seedlings were harvested and all morphological measurements taken initially were redone as well as root length (cm). Leaf area was also measured from five leaves per seedling using a LI-COR 3100 leaf area meter (LI-COR Inc., Lincoln, NE, USA). Harvested seedlings were dried at 70 °C for 48 hours, and leaf dry mass from five leaves (mg), shoot dry mass (mg), and root dry mass (mg) were obtained. SLA was calculated as leaf area divided by leaf dry mass using those five leaves.

Analysis of anthocyanin content

To examine the amount of anthocyanin produced in response to the three UV light treatments, one leaf from each seedling was weighed (taken from the third node from the tip of the main stem). At most, 10 seedlings/population/UV light level were used for anthocyanin measurement, depending on the number of seedlings that had survived at the end of the experiment. The relative amounts of anthocyanin were determined following the protocol used by Neff and Chory (1998) with some modifications. Leaf samples were homogenized in Lysing Matrix A tubes with the FastPrep-24 Instrument for 40 seconds at 4.0 m/s (MP Biomedicals, Santa Ana, CA, USA) after incubating overnight with 300 μ L of methanol with 1% HCl. Anthocyanins were separated by centrifuging with an additional 200 μ L distilled water and 500 μ L chloroform and transferred to a qubit tube. The relative amount of anthocyanin was determined by calculating the difference between the A_{530} and A_{657} readings per gram of fresh weight (Cominelli et al. 2008) of the aqueous phase measured by a spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Stress response tests in the growth chamber

To understand the environmental factors responsible for local adaptation, short-term stress responses of the seedlings to high-intensity UV radiation and low temperature were examined through growth-chamber experiments. At least 10 seedlings from each family were transplanted from the germination trays to individual 5 \times 5.5-cm pots at least three months after the seedlings were transferred to the second greenhouse and grown in the same greenhouse (i.e., visible light = \sim 700 μ mol/m²/s; and UV radiation = \sim 35 μ mol/m²/s) for approximately one year.

- UV radiation

Two seedlings were selected from one parental tree from each of the six elevations; one for treatment and another as a control. Seedlings of similar sizes were chosen; otherwise selection was haphazard within families. Seedlings were transferred into the growth chamber four days before the experiment at 22 °C with 40 μ mol/m²/s white light for 12-h/day. The six seedlings (one seedling/tree \times 6 source populations) were exposed to UV light with 80 μ mol/m²/s for 10-h/day, in addition to white light at 22 °C, until all seedlings died. Time to damage and death was recorded. The experiment was repeated in triplicate.

- Low temperatures

To examine the effect of low temperature, seedlings from each of the six source populations were tested in response to freezing and chilling temperatures. Prior to use in the experiment, seedlings grown in the second greenhouse were acclimatized at 22 °C with 40 μ mol/m²/s white light for 12-h/day for four days in the growth chamber. The temperature was dropped to 14 °C during the day time to simulate the maximum day-time temperature at the highest-elevation collection site (population) in winter. Annual mean temperature recorded from 1950-2000 was lowest in February at 2300 m above sea level on Hawai'i Island, ranging from 4-14 °C based on Global Climate Data (<http://www.worldclim.org/formats>). Three similarly sized seedlings were haphazardly chosen per family. Two seedlings were exposed to freezing and chilling temperatures for

10-h/day (~ -5 °C and ~ 4 °C, respectively), and the third seedling was kept in the growth chamber at 14 °C all day as control. The light cycle was identical across the three treatments (i.e. white light for 12-h and dark for 12-h/day). Survivorship and any signs of damage in response to low temperatures were observed for four days. The experiment was repeated four more times. In each trial, the degree of damage was recorded using the following scores: 3) seedlings were alive with no signs of damage, 2) seedlings were alive with signs of damage, 1) seedlings were dying, and 0) seedlings were dead. The experiment was repeated (n = 8).

Data analysis

For the reciprocal transplant experiment, survivorship was recorded as “1” and “0,” indicating living and dead seedlings, respectively. Since seedlings were outplanted at 90, 275, 1575, and 2300 m in elevation earlier than those at 950 and 2050 m, survivorship data 10 months after outplanting were used for analysis. The significant explanatory variables to predict survivorship were selected via a generalized linear model (GLM) analysis (McCullagh & Nelder 1989) with the binomial distribution among population, transplanted sites, and the interaction between population and outplanting sites in the “R” software (Ihaka and Gentleman 1996). A principal component analysis (PCA) was performed using the seedling measurements taken before the experiment using a software, PAST 3.06 (Hammer et al. 2001) to allow testing of the importance of initial seedling size for survivorship. To assess maternal effects, family was included as a random factor in all models, nested within population. The effect of plot nested within outplanting site was also investigated as a random factor. The best-fitting model was determined using stepwise model selection based on the Akaike information criterion (AIC).

Based on measurements taken at the end of the experiment, total photosynthetic area (TPA) was calculated using the following equation:

$$\text{TPA} = (\text{leaf width} / 2) \times (\text{leaf length} / 2) \times \pi \times \text{number of leaves}$$

To assess plant growth, a common formula to calculate a mean relative growth rate (MRGR) was used as follows (Poorter 1989):

$$\text{MRGR} = (\ln(M2) - \ln(M1)) / t,$$

where $M1$ and $M2$ are measurements at the beginning and end of the experiment, respectively, and t is the experiment period. GLM with Gaussian error structure was used to model the effects of population, family nested within population, and each of three PCA scores of initial seedling size on MRGR of main stem length and TPA of seedlings.

For the UV light experiment in the greenhouse, both survivorship and growth data were analyzed as a factorial with 6 elevations \times 3 UV light levels plus interactions, with initial seedling size (PCA scores) used as covariates. For survivorship, PCA scores were obtained from all of the initial seedling measurements and two highest scores were used to represent initial seedling size. The effects of explanatory factors on seedling survivorship in response to three UV light levels were modeled by GLM assuming a binomial construction. Since two seedlings were used per family for the experiment, the effect of seedlings nested within family was analyzed as a random factor.

Timing of pubescence production on seedlings was analyzed with factors including elevations, UV levels, interactions, and the initial seedling size shown as the same PCA 1 and 2 scores used above by using Cox Proportional Hazard (Cox PH) model. Quality of the models were assessed by AIC.

To analyze seedling growth (total dry mass, MRGR of main stem length/TPA, specific leaf area (SLA), root:shoot length ratio, and root:shoot dry mass ratio), values were averaged between the two seedlings per family. PCA scores for the initial seedling size were re-obtained from the seedling measurements averaged between two seedlings per family, and the two highest scores were used for analyses. Specific leaf area (SLA) was calculated from five leaves collected per seedlings from the third node from the tip of the main stem. Values for anthocyanin content were log-transformed because the data were not normally distributed. For anthocyanin content, PCA scores for the seedling size before the experiment were obtained from seedlings used for the analysis. GLM with a Gaussian distribution was used for analyses of seedling growth and anthocyanin content. Considering the data were over- or less-dispersed, the significance of each variable was also calculated by deletion tests by comparing a full model to a model with only one term.

To analyze stress responses to UV radiation, times to damage and death were recorded. Since no control seedlings died during the experiment, times to damage and death for treated seedlings were analyzed by one-way ANOVAs.

For the test of response to low temperatures in the growth chamber, the damage levels were recorded as 3 = alive without any damage, 2 = damaged (partially drying, withered, black leaves), 1 = dying (starting drying more than half of a seedling), and 0 = dead. The damage level scores were averaged among seedlings from the same source population each day. Damage levels at Day 4 were evaluated by two-way ANOVAs including interaction terms with population and treatment as factors.

Results

Reciprocal transplant experiment

There were strong differences in survival of outplanted seedlings at the different sites. PCA1 of initial seedling morphology explained 75% of the total variance and comprised mainly the number of leaves; PCA2 explained 17% of the total variance and was most heavily weighted by length measures (i.e., leaf and root length; Table 2). The best-fit model to predict seedling survivorship after the 8-month reciprocal transplant experiment included outplanting site and the two PCA scores of initial seedling morphology (AIC = 766.31; AIC = 796.88 in the null model). The second best-fitting model (AIC = 768.62) included elevation, outplanting site, and the two PCA scores. The GLM analysis indicated significant effects of outplanting site ($\chi^2 = 183.49$; d.f. = 5; $p < 0.0001$), plots within site ($\chi^2 = 398.48$; d.f. = 41; $p < 0.0001$), and PCA 2 ($\chi^2 = 5.55$; d.f. = 1; $p < 0.0185$) on seedling survivorship (Table 3), suggesting that initial leaf length positively affected survivorship. Marginally significant effects of elevation (original) were also detected ($\chi^2 = 10.65$; d.f. = 5; $p = 0.0589$; Table 3). There were no significant effects of interactions involving original elevations and outplanting sites on survivorship (Table 3).

Survivorship of seedlings from all populations was very low at the two highest-elevation outplanting sites ranging from 0% (for seedlings derived from 90-m and 950-m elevations) to 10% (for 275-m and 2300-m seedlings) at the 2050-m planting site after 10-months, and from 10% (1575-m seedlings) to 30% (275-m seedlings) at the 2300-m outplanting site (Fig. 3). Seedling mortality slowed after four months of rapid decline at 2300 m, and after two months of severe mortality at 2050 m. In contrast, seedlings planted at a middle elevation, 1575 m, had high survivorship overall ranging from 65% (90-m seedlings) to 90% (275-m seedlings). Seedlings from lower elevations had relatively higher survivorship at low elevations than seedlings from higher elevations, especially at the 90-m and 275-m sites. The range in survivorship was wider at the two lowest elevations, ranging from 5% (1575- and 2300-m seedlings) to 45% (950-m seedlings) at the 90-m site, and from 5% (1575-m seedlings) to 40% (275-m seedlings) at the 275-m site. Survivorship at the 950-m site was similar across all outplanted seedlings and fell between 35-60 % at 10 months. Interestingly, seedlings derived from high-elevation seeds did not show relatively greater survivorship at higher elevations compared to seedlings from lower elevations at 10 months. At the 275-m site, survivorship of home seedlings was highest after six-, seven-, eight-, nine, and 10-months of the experiment. According to GLM to compare survivorship between home seedlings and ones from other elevations at the 275 m, there was a significant difference in survivorship between home seedlings and 2-higher-elevation seedlings (1575 m, $z = -2.38$, $p = 0.0173$; 2050 m, $z = -2.38$, $p = 0.0171$; Table 4). Marginally significant differences in survivorship were found in home seedlings with 950-m seedlings ($z = -1.66$, $p = 0.0968$) and with 2300-m seedlings ($z = -1.69$, $p = 0.0916$). There was no significant difference between home seedlings and lowest-elevation seedlings at the 275-m site ($z = -0.78$, $p = 0.4361$). Although seedlings planted at 90 m were not exposed to a full natural condition because they were covered with a shade cloth during the experiment, there was a trend of relatively higher survivorship of lower elevation seedlings.

MRGR was calculated for seedlings outplanted at 1575 m to see if there is a difference in plant growth among seedlings from the six elevations, since overall seedling survivorship was high at the 1575-m site. PCA1 re-obtained based on the initial measurements for seedlings planted at 1575 m accounted for 72% of the total variance and again was loaded most heavily with number

of leaves (Table 5). PCA2 accounted for 13% of the total variance and comprised mainly leaf length. PCA3 explained 10% and was loaded most heavily with root length. Based on the GLM, there were no significant factors that influenced the MRGR of either main stem length or TPA (Table 6). Only when the MRGR of main stem length was compared between seedlings from 1575 m and 2300 m was there a marginally significant difference ($t = -1.713$; $p = 0.0930$). Although not statistically significant, the MRGR of main stem length was highest in seedlings planted at their home elevation (1575 m; Fig. 4), and these plants also had relatively high MRGR of TPA at the 1575-m site (Fig. 5).

Using the HOBO Weather Stations, temperature, RH, and PAR were successfully measured from March 2014 to April 2015 at 90, 1575, and 2300m. At 275 m, temperature and RH were recorded from September 2014 to April 2015, and PAR data were not available due to equipment failure. At 950 m, temperature, RH, and PAR began recording from December 2014. Although a weather station was placed at 2050 m, no data were obtained due to technical problems. Mean monthly temperatures decreased with elevation with the exception of high temperatures at 2300 m between September to November in 2014 (Fig. 6). Mean RH was highest at middle elevations (950 and 1575 m) and lowest at 2300 m (Fig. 7). Mean RH was low during winter, especially at 2300 m. Mean monthly PAR as highest at 2300 m for all months except September 2014, while mean PAR was low at middle elevations (950 and 1575 m; Fig. 8). Mean PAR was low during winter and high during summer.

UV light experiment in the greenhouse

Significant variation in survivorship was observed among seedlings from the different elevations at the end of the 12-month greenhouse UV-light experiment. PCA1 (strong loading of number of leaves) and PCA2 (strong loading of leaf length) accounted for 79% and 19% of the total variance, respectively (Table 7). According to the stepwise model selection, the best model was a full model including all variables. The effects of original elevation ($\chi^2 = 237.37$; d.f. = 5; $p < 0.0001$), the interaction between UV level and elevation ($\chi^2 = 40.32$; d.f. = 10; $p < 0.0001$), and PCA1 score ($\chi^2 = 32.01$; d.f. = 1; $p < 0.0001$) were significant on seedling survivorship in the greenhouse (Table 7). UV light levels ($\chi^2 = 5.97$; d.f. = 2; $p = 0.0505$) and PCA2 score ($\chi^2 = 2.89$; d.f. = 1; $p = 0.0894$) had marginally significant effects on survivorship. Survivorship varied significantly between seedlings within families ($\chi^2 = 512.57$; d.f. = 239; $p < 0.0001$). Overall, seedlings from lower elevations had higher survivorship than seedlings from higher elevations (Fig. 9). GLMs were also performed for the three lower elevation populations and the three higher elevation populations separately, because there was a difference in the pattern of seedling survivorship between those two groups. Survivorship of seedlings from the lower elevations was influenced by all factors, while survivorship of seedlings from higher elevations was influenced by all factors except the interaction between UV light level and elevation and seedling within family (Table 8). There was a trend toward high UV promoting survivorship of seedlings from lower elevations, and suppressing survivorship of seedlings from higher elevations, although it was not statistically significant. Survivorship was higher under ambient UV than under no UV for all populations except for seedlings from the lowest and highest elevations (90 and 2300 m, respectively). The maximum (40-54 °C) and minimum (14-20 °C) temperatures were relatively consistent among UV light levels.

Stepwise model selection showed all variables, except PCA2 score, to be important in the prediction of the timing of pubescence production (AIC = 5117.53; AIC = 5119.04 in the null model). According to the Cox PH model which is also called survival analysis, modeling the time taken for events to occur, the timing of pubescence production was significantly affected by elevations ($\chi^2 = 38.25$; d.f. = 5; $p < 0.0001$), PCA1 score ($\chi^2 = 19.47$; d.f. = 1; $p < 0.0001$), and the interaction between elevations and UV levels ($\chi^2 = 20.83$; d.f. = 10; $p = 0.0223$; Table 9). Pubescence started to appear on seedlings 3.5-4 months after the experiment started in the greenhouse and became prevalent across seedlings five months into the experiment (Fig. 10). Seedlings from the three lower elevation populations produced pubescence faster than seedlings from the three higher elevation populations under all three UV levels. However, the contrasting timing of pubescence development between lower and higher elevations was greatest under extra UV light and smallest under no UV light.

At the end of the 12-month experiment, seedling dry mass was significantly affected by UV light level ($F = 14.33$; d.f. = 2; $p < 0.0001$), elevation ($F = 24.62$; d.f. = 5; $p < 0.0001$), the interaction between UV level and elevation ($F = 2.76$; d.f. = 10; $p = 0.0042$), and PCA 1 score ($F = 21.06$; d.f. = 1; $p < 0.0001$; Table 10). The number of leaves positively affected total dry mass. The mean total dry masses of seedlings from 90-950 m were relatively higher than those of higher elevation seedlings regardless of UV light levels (Fig. 11). Both high and ambient UV light suppressed the mean dry mass of seedlings from all elevations except those from 1575 m.

MRGRs of main stem length and TPA were significantly influenced by UV level and population (Table 11). No effect of the interaction between UV level and elevation was found on the MRGR of main stem length ($F = 1.34$; d.f. = 10; $p = 0.2158$), but a marginal effect of the interaction on MRGR of TPA was shown ($F = 1.76$; d.f. = 10; $p = 0.0761$). Across elevations, a similar pattern of MRGRs of both main stem length and TPA was revealed in seedlings in response to the three UV light levels; the three lower elevations had consistently higher growth rates regardless of UV light level, while the three higher elevations had decreased growth rates under UV light in a broad sense (Fig. 12 & 13). GLM analyses performed separately for the three lower and three higher elevation populations revealed different significant factors (Table 10). For seedlings from the three higher elevations, UV light level significantly influenced both MRGRs. Elevation was a significant factor influencing MRGR of TPA ($F = 9.08$; d.f. = 2; $p = 0.0017$), but had only a marginally significant effect on MRGR of main stem length ($F = 3.23$; d.f. = 2; $p = 0.0617$). For lower elevation seedlings, elevation significantly influenced MRGR of main stem length ($F = 5.73$; d.f. = 3; $p = 0.0012$), while UV level did not ($F = 0.15$; d.f. = 2; $p = 0.8607$); and MRGR of TPA was influenced by UV levels ($F = 4.46$; d.f. = 2; $p = 0.0140$) and the interaction between population and UV level ($F = 2.94$; d.f. = 4; $p = 0.0241$) with no elevation effect ($F = 1.61$; d.f. = 3; $p = 0.1926$).

Elevation was a significant factor influencing SLA ($F = 3.40$; d.f. = 5; $p = 0.0045$), but UV light level did not differentiate SLA in seedlings ($F = 1.20$; d.f. = 2; $p = 0.3010$; Table 12). SLA under high UV was relatively higher in seedlings from lower elevations (Fig. 14).

The mean root:shoot dry mass ratio varied significantly across seedlings from the six populations ($F = 12.96$; d.f. = 5; $p < 0.0001$) but not across the three light levels ($F = 1.39$; d.f. = 2; $p = 0.2541$; Table 13). For the mean root:shoot length ratio, UV light level ($F = 18.7$; d.f. = 2; $p < 0.0001$) and elevation ($F = 3.83$; d.f. = 5; $p = 0.0030$) showed significant effects, and the interaction term was marginally significant ($F = 1.79$; d.f. = 10; $p = 0.0704$; Table 13). Seedlings had longer roots relative to stems under both UV light treatments for all elevations except 1575 m

under high UV (Fig. 15), which showed higher allocation to roots in terms of length. For dry mass, such a pattern of higher biomass allocation to roots was found in seedlings from 950 and 1575 m under both extra and ambient UV although the degree was different (Fig. 16).

For seedlings used for the anthocyanin content analysis a PCA of their initial morphology measurements was done. PCA1 (primarily shoot-length) explained 74% of the total variance, and PCA2 (number of leaves) accounted for 18% (Table 14). There was a significant difference in anthocyanin content among UV levels ($F = 8.38$; d.f. = 2; $p = 0.0006$) and elevations ($F = 2.69$; d.f. = 5; $p = 0.0293$; Table 15). The effect of interaction between UV levels and elevations was not significant ($F = 1.20$; d.f. = 10; $p = 0.3067$). An increase in anthocyanin content was observed under extra and ambient UV only except under ambient UV for 1575-m seedlings (Fig. 17).

In the analyses with all six elevations, initial seedlings size showed a significant impact (as a covariate) on seedling survivorship. Initial seedling size was also a significant covariate in the analyses of the timing of pubescence production (PCA 1 score), total seedling dry mass (PCA 1 score), and MRGRs of main stem length and TPA (PCA 1 and 2 scores). Maternal tree effects tested as mother trees nested within elevations in GLMs were found for MRGRs of main stem length and TPA including all elevations, mean root:shoot length and mean root:shoot dry mass ratio.

A high level of thrip damage was found in seedlings from lower elevations under ambient UV, whereas very few seedlings were damaged by thrips under extra UV (Fig. 18). No thrip damage was observed under no UV.

Stress response tests in the growth chamber

● UV radiation stress

Time to damage of treated seedlings ranged from four days (for seedlings from 1575 and 2050 m) to 24 days (for a seedling from 2300 m). As a trend, the mean time to damage was positively related to elevation, with the exception of 1575 m (Fig. 19). Seedlings from 1575 m responded relatively quickly to UV radiation, similar to seedlings from 90 m. There was no variation in the mean time to damage among elevations ($F = 0.5233$, d.f. = 5, $p = 0.7545$). Time to death of seedlings ranged from 12 days (for seedlings from 90 and 1575 m) to 64 days (for a seedling from 950 m). With elevations pooled into two groups (90-950 m and 1575-2300 m), the mean time to death tends to increase with elevation within each group (Fig. 20). No difference in the mean time to death was detected across elevations ($F = 1.0165$, d.f. = 5, $p = 0.4498$).

● Cold stress

After four days of exposure to low temperatures, no seedlings died in response to chilling at 4 °C for four days. In response to freezing at -5 °C for four days, all seedlings from the highest elevation survived, whereas seedlings from lower elevations had lower survivorship (Fig. 21). After exposure to -5 C for four days, seedling damage was significantly affected by treatment ($F = 188.09$, d.f. = 2, $p < 0.0001$), elevation ($F = 3.65$, d.f. = 5, $p = 0.0047$), and the interaction between treatment and elevation ($F = 2.63$, d.f. = 10, $p = 0.0075$). The mean damage level was relatively greater in seedlings from lower elevations over time, indicating seedlings from lower elevations were more sensitive to freezing temperature (Fig. 22).

Discussion

*Local adaptation of *Metrosideros polymorpha* across elevations*

The purpose of this study was to understand what role elevation plays in divergence within the tree species *Metrosideros polymorpha* through examination of fine-scale local adaptation across elevations and the effects of environmental factors on seedling survivorship and growth. The findings show evidence of home-site advantage in pubescent forms of *Metrosideros polymorpha* at the variety level along an elevation gradient with weaker evidence of population-level local adaptation. Further, results from the UV light experiment in the greenhouse and stress response tests in the growth chamber suggest the importance of UV radiation and temperature as drivers of local adaptation in trees across long elevation gradients.

VARIETY-LEVEL ADAPTATION

Local adaptation at the variety level

The combined experiments in this study support the separation of pubescent populations of *M. polymorpha* on Hawai'i Island into two varieties, var. *incana* at low elevations (i.e., the three lowest elevations included in this study) and var. *polymorpha* at high elevations (the two highest elevations included in this study); trees at the remaining population (1575 m elevation) may be inter-varietal hybrids (see below). The relatively higher survivorship of var. *incana* seedlings within their home range (from sea level to 900 m; Johansen, pers. obs.) in the reciprocal transplant experiment indicate that var. *incana*, is adapted to its local environment. Local adaptation of the high-elevation variety, var. *polymorpha*, was not as clear given the overall low survivorship of outplanted seedlings at the two highest elevations, probably due to low habitat quality. According to data from a HOBO weather station positioned at 2300 m, the average temperature was lowest most of the time during the reciprocal transplant experiment, relative humidity was also lowest, and PAR was highest compared to other outplanting sites. Soil moisture was not measured in this study, but low relative humidity suggested the 2300-m site was dry. Limited water availability was likely an important cause of seedling death at high elevation, as drought is a major determinant of plant performance (Rivero et al. 2007). In fact, summer drought was the main factor to cause seedling death (86.5 %) over winter frost (10.5 %) and damages by pathogen/herbivore (3 %) in the high-elevation Mediterranean plant (*Silene ciliata*; Gimenez-Benavides et al. 2007). Therefore, dry conditions at 2300 m may be a leading reason why seedling survivorship was very low. Earlier studies documented low performance of plants outplanted outside of their home ranges (Link et al. 2003; Angert & Schemske 2005). Given that lower survivorship was found in high-elevation seedlings planted at lower elevations compared to survivorship in low-elevation seedlings in this study, we can say at least that var. *polymorpha* is poorly adapted to low elevations.

*Different responses by *M. polymorpha* vars. *incana* and *polymorpha* to UV radiation*

Both the long-term greenhouse experiment and the short-term stress response test to UV radiation suggested an ecological difference between vars. *incana* and *polymorpha* in response to

UV radiation. Responses to UV radiation in the greenhouse correspond clearly to two varieties: UV radiation positively correlates with survivorship of seedlings from lower elevations, while UV radiation negatively correlates with survivorship of seedlings from higher elevations, suggesting better acclimatization to UV radiation in seedlings from lower elevations in the greenhouse conditions. Given that UV radiation generally increases with increasing elevation, seedlings at high elevations were predicted to show greater adaptation to high UV radiation. However, the results from the greenhouse experiment revealed the opposite pattern (although high-elevation seedlings showed greater anthocyanin accumulation in leaves in response to high UV; see below). One possible explanation for this pattern is that seedlings from lower elevations had acclimatized to UV radiation more efficiently in the greenhouse conditions because the greenhouse is located at 100 m. This scenario is probably supported by MRGRs in seedlings. MRGRs of main stem length and TPA were consistently high in seedlings from lower elevations, while UV levels negatively affected MRGRs in seedlings from higher elevations. This study is another example indicating that sensitivity to UV radiation is not always found in plants from lower elevations (Barnes et al. 1987; Rau & Hafmann 1996).

The short-term stress test showed an increase in the time to damage in response to UV with elevations, except for a relatively fast response in seedlings from 1575 m. On the other hand, time to death increased with elevations within each variety. Thus, in terms of their short-term response to UV radiation, lower elevation populations were more sensitive to UV radiation within a variety. Although there are differences in patterns between short- and long-term responses to UV in seedlings, the short-term UV exposure also distinguished two pubescent varieties.

Inter-relationship between plants and herbivores under UV radiation (biotic and abiotic stress)

A high level of thrip damage was found in seedlings under ambient UV radiation especially from lower elevations, although only a few seedlings were damaged by thrips under extra UV radiation. High UV radiation can prevent herbivore damage on plants directly by affecting insects and indirectly by stimulating compounds in plants in response to UV radiation (Kuhlmann & Müller 2011). There are negative effects of UV radiation on insects, and decreased survivorship of insects under UV radiation has been reported (McCloud & Berenbaum 1994; Bacher & Luder 2005; Ohtsuka & Osakabe. 2009). In addition, it is documented that thrips (*Caliothrips phaseoli*) can visually detect UV-B radiation (Mazza et al. 2010). Although the thrips species that damaged *M. polymorpha* in the greenhouse was not identified, it is possible that it is deterred by high UV radiation.

Some signaling pathways are the same for UV protection and herbivore defense (Kuhlmann & Müller 2011). For example, flavonoids are a well described family of compounds that plants produce for protection against UV radiation (Barnes et al. 1987; Ziska et al. 1992), and against herbivores (Ho et al. 2003). Anthocyanin is a type of flavonoid that are recognizable as UV-absorbing pigments derived from phenylalanine (Li et al. 1993). There was no clear correlation between anthocyanin production and the level of thrip damage in this study. However, it is

possible that seedlings produced other protective compounds in response to UV radiation, which may have prevented thrip damage under extra UV radiation.

Under ambient UV, thrip damage was seen almost exclusively on seedlings from the three lowest elevations (var. *incana*). Thrips may have been attracted to lower elevation seedlings because plants were bigger and had more fresh leaves, or thrips in the greenhouse may have been better adapted to feed on seedlings of var. *incana*.

Thrip damage may have caused a decline in seedling survivorship and growth because there was an inverse relationship between the proportion with thrip damage and seedling survivorship/total dry mass. As a possible explanation, growth-regulating hormone may affect this relationship. It has been described that synthesis of the growth-regulating hormone, auxin, is reduced under UV-B radiation as a stress response, and decreased auxin can act as defense against herbivore damage (for a review, see Kuhlmann & Müller 2011). Finally, the lack of thrip damage in the absence of UV was likely due to the polycarbonate panel that partially covered the seedlings and that may have blocked access to seedlings by thrips.

Different responses by M. polymorpha vars. incana and polymorpha to temperature

Seedlings from the three highest-elevation populations were more capable of surviving exposure to four days of freezing temperatures, while seedlings from lower elevations were sensitive to freezing temperature. It is not surprising because plants from high elevations are expected to be adapted to low temperatures. The capacity for leaf expansion was reported in *Poa* species from high elevation exposed to freezing temperature (Korner & Woodward 1987). Furthermore, phylogenetic relationships show that Hawaiian *Metrosideros* is derived from New Zealand *Metrosideros*, including *M. umbellata*, which is frost-tolerant species at the base of the clades in the Pacific islands (Wright et al. 2000 and 2001). Therefore, *M. polymorpha* at high elevation may have retained ancestral genes for freezing tolerance, which may explain why seedlings even from low elevations were capable of surviving chilling temperature.

The long-term greenhouse experiment strongly suggested that seedlings from high elevations are sensitive to heat because seedling survivorship and growth were relatively higher in seedlings from lower elevations compared to the ones from higher elevations. High temperature tolerance may be an important factor to distinguish these varieties. Previous studies suggested that plants from high elevations are sensitive to high temperature (Angert & Schemske 2005; Gimenez-Benavides et al. 2007). According to data from HOBO weather stations, average temperatures in the field were approximately 10-17 °C and 22-25 °C at higher elevations (1575 and 2300 m) and lower elevations (90 and 275 m), respectively. The greenhouse is located at ~100 m in elevation, and the maximum temperature to which seedlings were exposed in this experiment reached over 50 °C. The effects of high temperature on the survivorship of seedlings from the six elevations should be examined directly.

POPULATION-LEVEL ADAPTATION

Local adaptation at a fine scale

Results from these experiments, including the reciprocal transplant experiment 10 months after outplanting, may suggest local adaptation of *M. polymorpha* at the population level, including differential local adaptation within var. *incana*. At 10 months, survivorship of outplanted seedlings was greatest at “home” only at the 275-m outplanting site. At this site, survivorship of seedlings from 90 m was not significantly different, whereas survivorship of all seedlings from elevations above 275 m was significantly (or marginally significantly) lower. This result may suggest differential local adaptation within var. *incana* with a separation at roughly 275 m. Similarly, the greater MRGR of stem length of seedlings outplanted at home at 1575 m may also suggest local adaptation at the population level. Interestingly, seedlings from 1575 m had very low survivorship at all other outplanting sites except 950 m, where mean relative humidity was even higher than that at 1575 m. Further, the unique response of seedlings from 1575 m to UV light in the 12-month greenhouse experiment (i.e., greater total dry mass under ambient UV than under extra or no UV) may be consistent with adaptation to persistent cloud cover, which reduces UV flux (Korner 2007). The high sensitivity of seedlings from 1575 m to UV radiation in the short-term stress response experiment supports this idea. These observations combined suggest that *M. polymorpha* from 1575 m may be locally adapted to a uniquely wet environment. Given the extended lifespan of *M. polymorpha*, a longer observation period in the reciprocal outplanting experiment will be required to determine if evidence of population-level local adaptation will persist at these and other elevations. Given the extreme mortality observed in seedlings outplanted at the two highest-elevation sites, local adaptation at the population level within var. *polymorpha* was not detectable.

Uniqueness (possible intervarietal hybrids) at upper-middle elevation

In the 12-month greenhouse experiment, seedlings from 1575 m behaved uniquely: seedlings from only 1575 m had higher total dry mass under ambient UV compared to that under extra and even no UV. The unique behavior of seedlings from 1575 m may suggest that trees at 1575 m are hybrids among the two pubescent varieties *incana* and *polymorpha* as well as the glabrous variety *glaberrima*. Varieties of *M. polymorpha* are known to hybridize where they co-occur (Stacy et al., in review; pers. obs.). The reciprocal transplant experiment showed high survivorship of seedlings from all elevations at 1575 m, suggesting that the environment at this elevation is optimal for seedlings of both varieties as well as their hybrids. A HOBO weather station at 1575 m recorded high relative humidity, ranging from 89-97%. Given that drought has severe effects on plants (Rivero et al. 2007), a wet environment can provide high quality habitat for plants.

UV PROTECTIVE COMPOUND

Difference in anthocyanin accumulation under UV light levels

The level of anthocyanin in leaves was consistently low under no UV radiation at the end of the 12-month light experiment in the greenhouse, indicating no differences in anthocyanin accumulation among populations across elevations without UV radiation. In contrast, there was a positive relationship between UV light intensity and anthocyanin accumulation across elevations, suggesting that UV radiation stimulated anthocyanin accumulation in all populations. Plants exposed to high UV flux can be protected from UV damage without the additional accumulation of protective compounds (Barnes et al. 1987; Ziska et al. 1992). The current study, however, failed to show a clear evidence of fixed adaptation of any population or variety to UV radiation. Further, anthocyanin content was not always highest under extra UV compared to that under ambient and no UV, although it increased with UV light levels in seedlings from the two highest elevations. High variation in anthocyanin content among low-elevation seedlings may appear because seedlings from lower elevations do not need to maintain efficient protection against UV radiation as shown in arctic plants under low UV-B flux (Robberecht et al. 1980).

Relationship between UV-protective compounds and plant growth

Across all elevations in the 12-month light experiment, an inverse relationship was found between anthocyanin production and total dry mass. Anthocyanin is synthesized in response to abiotic stress such as UV radiation and its metabolic cost is high because additional chemical modification is required to produce anthocyanin (Chalker-Schott 1999; Paul et al. 2012). The result from the greenhouse suggested that there is trade-off between anthocyanin production and plant growth. Other phenolic compounds can be produced as UV protection, including other flavonoids, tannins, lignins and furanocoumarins (Kuhlmann & Muller 2011). A decline in seedling growth (e.g., total dry mass) can be associated with production of these UV-protective compounds.

Conclusions

These experiments document differential local adaptation between the two dominant pubescent varieties of the landscape-dominant tree, *M. polymorpha* on Hawaii Island and suggest population-level local adaptation at 275 m and possibly 1575 m along a long elevation gradient. Results of the greenhouse experiment suggested that UV radiation plays an important role in distinguishing the two varieties in terms of plant survivorship, thrip damage, and production of anthocyanin in response to high UV. High UV radiation promoted the survivorship of seedlings from lower elevations, but suppressed survivorship of higher elevation seedlings, and a majority of the thrip damage was found on lower elevation seedlings under ambient UV radiation. The short-term response experiments involving UV radiation and freezing also revealed variety-level adaptation. High temperature may also play a role in differentiating vars. *incana* and *polymorpha*, though confirmation through direct study is necessary. These results reveal insights into the role of elevation gradients in population divergence in trees as well as important ecological differences between two dominant forms of *M. polymorpha* on Hawai'i Island.

List of tables

Table 1. Description of six seed collection sites on East Hawai‘i Island. Varieties were identified based on morphological characteristics. Although seedlings from 1575 m were characterized as *M. polymorpha* var. *polymorpha*, they had notably longer internodes compared to trees from the two highest elevations.

Site Name	Variety	Volcano	Elevation (m above sea level)	Forest Type	GPS Coordinates
Paradise Park	<i>incana</i>	ML	88	Dry	19.5883-154.9939
South Puna	<i>incana</i>	ML	273	Mesic	19.4231-154.9493
Ka`u Desert	<i>incana</i>	KIL	951	Dry	19.3686-155.3673
Stainback High way	<i>polymorpha</i>	ML	1575	Cloud	19.5543-155.2962
Mauna Loa Strip Road	<i>polymorpha</i>	ML	2050	Subalpine Dry	19.4939-155.3852
Mauna Loa Access Road	<i>polymorpha</i>	ML	2300	Subalpine Dry	19.6155-155.4686

Table 2. Eigenvalues of seedling traits in first two PCA axes, obtained from the initial seedling measurements before the reciprocal experiment. The highest eigenvalues for each PCA axis are identified in bold. Values in parentheses indicate the percentage of variance explained by the two axes.

Traits	PCA 1 (74.53%)	PCA 2 (16.71%)
Number of stem	0.0735	-0.0446
Main stem length	0.1242	0.1991
Cumulative stem length	0.3330	0.1356
Number of leaves	0.8750	-0.3975
Leaf length	0.1832	0.5804
Leaf width	0.1103	0.3296
Root length	0.2388	0.5801

Table 3. Generalized Linear Model performed to explore effects of variables on seedling survivorship after the 10-month reciprocal transplant experiment. Note: × = interaction; () = nested within.

Variables	d.f.	χ^2	<i>P</i>
Elevation	5	10.65	0.0589*
Site	5	183.49	< 0.0001
Population × Site	25	30.85	0.1942
PCA1 initial seedling size	1	0.59	0.4440
PCA2 initial seedling size	1	5.55	0.0185
Mother (Elevation)	119	121.26	0.4253
Plot (Site)	41	398.48	< 0.0001

Note: Bold *p*-values are significant. * Marginally significant values.

Table 4. Generalized Linear Model performed to explore effects of variables on seedling survivorship after the 10-month reciprocal transplant experiment at the 275-m site.

Variable	Estimate	SE	Z	P
90-m seedlings	-0.55	0.71	-0.78	0.4361
950-m seedlings	-1.27	0.77	-1.66	0.0968*
1575-m seedlings	-2.76	1.16	-2.38	0.0173
2050-m seedlings	-2.2	0.92	-2.38	0.0171
2300-m seedlings	-1.28	0.76	-1.69	0.0916*

Note: Bold *p*-values are significant. * Marginally significant values.

Table 5. Eigenvalues of seedling traits in the first three PCA axes, obtained from the initial seedling measurements before outplanting at 1575 m. The highest eigenvalues for each PCA axis are identified in bold. Values in parentheses indicate the percentage of variance explained by the three axes.

Traits	PCA 1 (71.57%)	PCA 2 (12.52%)	PCA 3 (10.32%)
Number of stem	0.0756	0.0047	-0.0476
Main stem length	0.0682	0.1439	0.0847
Cumulated stem length	0.1025	0.0994	-0.0087
Number of leaves	0.9065	-0.2845	-0.2925
Leaf length	0.1840	0.8400	-0.1843
Leaf width	0.0809	0.4014	-0.1859
Root length	0.3420	0.1478	0.9145

Table 6. Generalized Linear Model performed to explore effects of variables on MRGR of main stem length at 1500 m after the 12-month reciprocal transplant experiment. () = nested in.

	<i>dfn</i>	<i>dfd</i>	<i>F</i>	<i>P</i>
MRGR of main stem length				
Elevation	5	58	0.9757	0.4420
PCA1	1	57	0.0795	0.7792
PCA2	1	56	0.5116	0.4778
PCA3	1	55	1.0067	0.3206
Mother(Elevation)	6	49	0.2901	0.9389
MRGR of TPA				
Elevation	5	58	0.3252	0.8954
PCA1	1	57	0.8596	0.3584
PCA2	1	56	0.0476	0.8283
PCA3	1	55	1.8140	0.1842
Mother(Elevation)	6	49	0.8087	0.5682

Table 7. Eigenvalues of seedling traits in two PCA axes, obtained from the initial seedling measurements before the 12-month greenhouse experiment. The highest eigenvalues for each PCA axis are identified in bold. Values in parentheses indicate the percentage of variance explained by the two axes.

Trait	PCA 1 (78.73%)	PCA 2 (18.59%)
Shoot length	0.0684	0.0549
Number of leaves	0.8281	-0.3960
Number of nodes	0.3743	-0.1315
Leaf width	0.1797	0.4009
Leaf length	0.3703	0.8138

Table 8. Generalized Linear Model performed to explore effects of variables on seedling survivorship after the 12-month greenhouse experiment with three UV light levels. Note: × = interaction; () = nested within.

Variable	d.f.	χ^2	<i>P</i>
All elevations			
UV level	2	5.97	0.0505*
Elevation	5	237.37	< 0.0001
UV level × Elevation	10	40.32	< 0.0001
PCA 1 initial seedling size	1	32.01	< 0.0001
PCA 2 initial seedling size	1	2.89	0.0894*
Seedling (Tree)	239	512.57	< 0.0001
3 lower elevations			
UV level	2	9.24	0.0099
Elevation	2	8.20	0.0165
UV level × Elevation	4	20.14	0.0005
PCA 1 initial seedling size	1	18.09	< 0.0001
PCA 2 initial seedling size	1	4.66	0.0309
Seedling (Tree)	119	152.26	0.0214
3 higher elevations			
UV level	2	10.29	0.0058
Elevation	2	9.47	0.0088
UV level × Elevation	4	7.22	0.1247
PCA 1 initial seedling size	1	15.10	0.0001
PCA 2 initial seedling size	1	1.46	0.2263
Seedling (Tree)	119	142.74	0.0682*

Note: Bold *p*-values are significant. * Marginally significant values.

Table 9. Cox Proportional Hazards Regression Model performed to explore the effects of variables on the timing of pubescence production during the 12-month greenhouse experiment with three UV light levels. Note: × = interaction.

Variable	d.f.	χ^2	<i>P</i>
UV level	2	0.74	0.6882
Elevation	5	38.25	< 0.0001
UV level × Elevation	10	20.83	0.0223
PCA 1	1	19.47	< 0.0001
PCA 2	1	0.22	0.6417

Note: Bold *p*-values are significant.

Table 10. Generalized Linear Model performed to explore the effects of variables on total seedling dry mass after the 12-month greenhouse experiment with three UV light levels. Note: × = interaction; () = nested within.

Variable	d.f.	<i>F</i>	<i>P</i>
UV level	2	14.33	< 0.0001
Elevation	5	24.62	< 0.0001
UV level × Elevation	10	2.76	0.0042
PCA 1 initial seedling size	1	21.06	< 0.0001
PCA 2 initial seedling size	1	1.00	0.3198
Mother (Elevation)	102	1.15	0.2263

Note: Bold *p*-values are significant.

Table 11. Generalized Linear Model performed to explore the effects of variables in MRGR of main stem length and total photosynthetic area (TPA) after the 12-month greenhouse experiment with three UV light levels. Note: × = interaction; () = nested within.

Variable	MRGR of main stem length			MRGR of TPA		
	d.f.	<i>F</i>	<i>P</i>	d.f.	<i>F</i>	<i>P</i>
6 elevations						
UV level	2	5.23	0.0066	2	16.88	< 0.0001
Elevation	5	29.54	< 0.0001	5	43.45	< 0.0001
UV level × Elevation	10	1.34	0.2158	10	1.76	0.0761*
PCA 1 initial seedling size	1	132.39	< 0.0001	1	296.74	< 0.0001
PCA 2 initial seedling size	1	14.89	0.0002	1	153.88	< 0.0001
Mother (Elevation)	102	1.43	0.0290	102	1.42	0.0334
3 lower elevations						
UV level	2	0.15	0.8607	2	4.46	0.0140
Elevation	3	5.73	0.0012	3	1.61	0.1926
UV level × Elevation	4	0.2	0.9353	4	2.94	0.0241
PCA 1 initial seedling size	1	133.16	< 0.0001	1	209.61	< 0.0001
PCA 2 initial seedling size	1	7.13	0.0089	1	112.45	< 0.0001
Mother (Elevation)	56	1.64	0.0158	56	1.24	0.1731
3 higher elevations						
UV level	2	6.93	0.0055	2	7.61	0.0037
Elevation	2	3.23	0.0617*	2	9.08	0.0017
UV level × Elevation	4	0.47	0.7558	4	73.36	0.3380
PCA 1 initial seedling size	1	11.98	0.0026	1	33.96	< 0.0001
PCA 2 initial seedling size	1	5.81	0.0263	1	1.21	< 0.0001
Mother (Elevation)	46	0.82	0.7558	46	1.36	0.2359

Note: Bold *p*-values are significant value. * Marginally significant values.

Table 12. Generalized Linear Model performed to explore the effects of variables on seedling SLA after the 12-month greenhouse experiment with three UV light levels. Note: × = interaction; () = nested within.

Variable	d.f.	<i>F</i>	<i>P</i>
UV level	2	1.20	0.3010
Elevation	5	3.40	0.0045
UV level × Elevation	10	0.64	0.7835
PCA 1 initial seedling size	1	1.20	0.2740
PCA 2 initial seedling size	1	0.68	0.4096
Mother (Elevation)	102	0.93	0.6695

Note: Bold *p*-values are significant.

Table 13. Generalized Linear Model performed to explore the effects of variables on seedling root:shoot dry mass/length ratio after the 12-month greenhouse experiment with three UV light levels. Note: × = interaction; () = nested within.

Variable	Root:shoot dry mass ratio			Root:shoot length ratio		
	d.f.	<i>F</i>	<i>P</i>	d.f.	<i>F</i>	<i>P</i>
UV level	2	1.39	0.2541	2	18.70	< 0.0001
Elevation	5	12.96	< 0.0001	5	3.83	0.0030
UV level × Elevation	10	4.16	0.0001	10	1.79	0.0704*
PCA 1 initial seedling size	1	0.14	0.7109	1	0.08	0.7744
PCA 2 initial seedling size	1	2.12	0.1479	1	0.00	0.9526
Mother (Elevation)	102	2.06	0.0001	102	1.40	0.0385

Note: Bold *p*-values are significant. *Marginally significant.

Table 14. Eigenvalues of seedling traits in two PCA axes, obtained from the initial measurements before the experiment in the greenhouse for seedlings used for anthocyanin content analysis. The highest eigenvalues for each PCA axis are identified in bold. Values in parentheses indicate the percentage of variance explained by the two axes.

Traits	PCA 1	PCA 2
	(73.57%)	(17.73%)
Shoot length	0.7545	-0.6030
Number of leaves	0.5042	0.7363
Number of nodes	0.2476	0.3061
Leaf width	0.1566	0.0068
Leaf length	0.3011	0.0227

Table 15. Generalized Linear Model performed to explore the effects of variables on anthocyanin content after the 12-month greenhouse experiment with three UV light levels. Note: × = interaction; () = nested within.

Variable	d.f.	<i>F</i>	<i>P</i>
UV level	2	8.38	0.0006
Elevation	5	2.69	0.0293
UV level × Elevation	10	1.20	0.3067
PCA 1 initial seedling size	1	2.52	0.1176
PCA 2 initial seedling size	1	0.62	0.4345
Mother (Elevation)	55	0.91	0.6522

Note: Bold *p*-values are significant.

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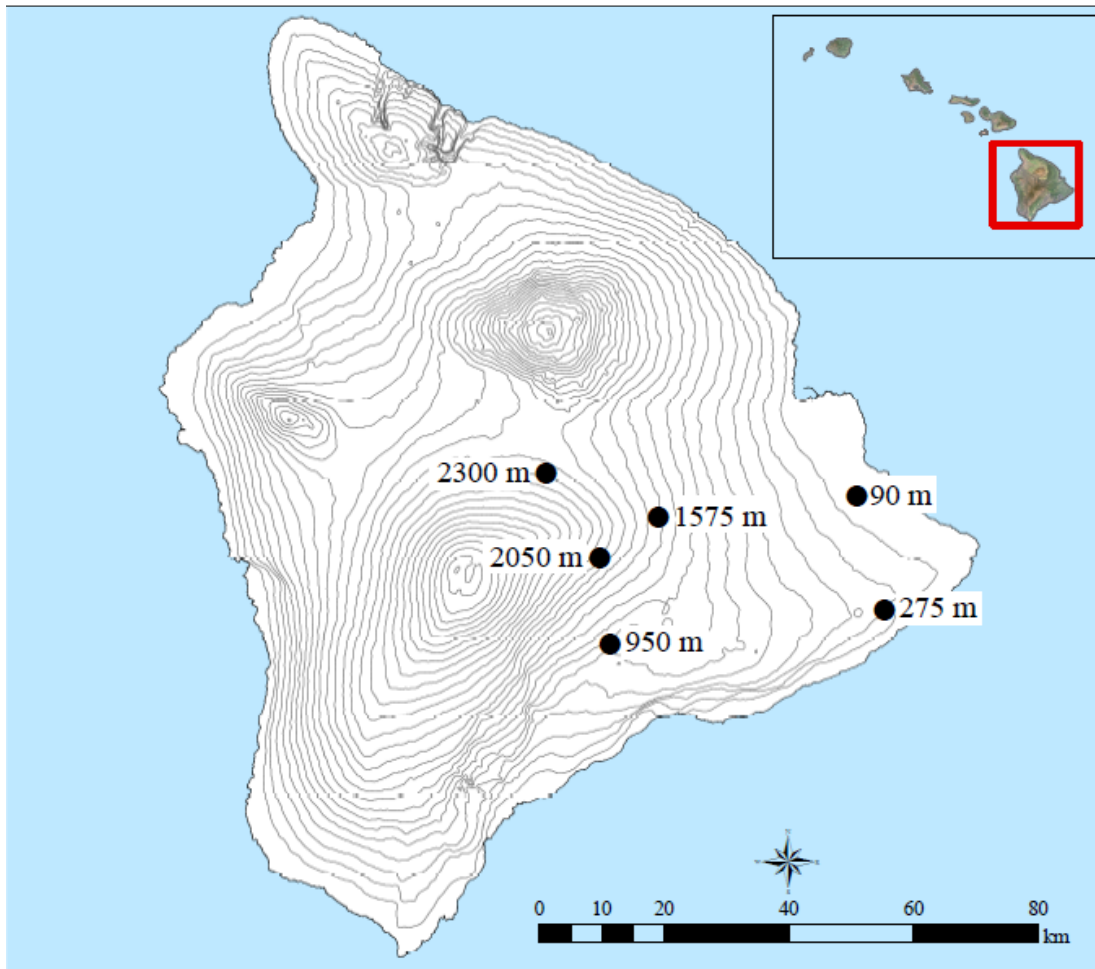


Fig. 1. Map of seed collection sites on Hawai'i Island. Seeds were collected from six elevations.

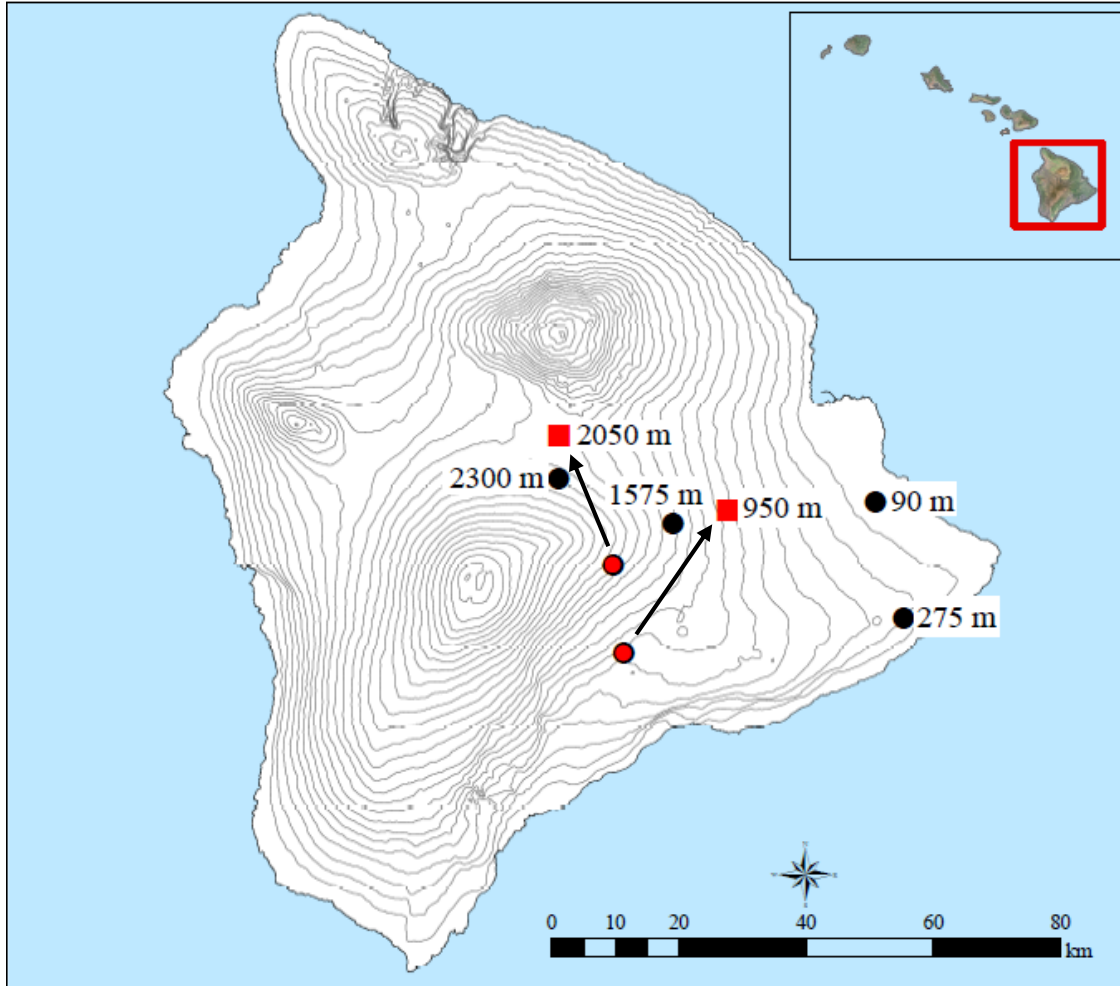
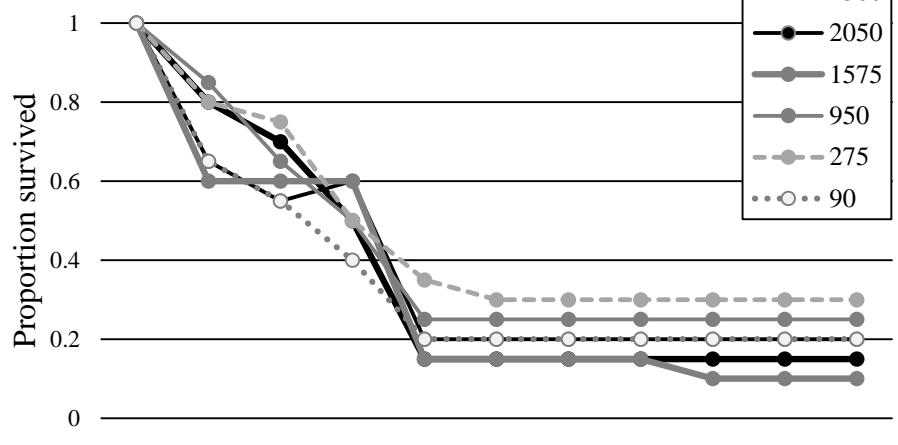
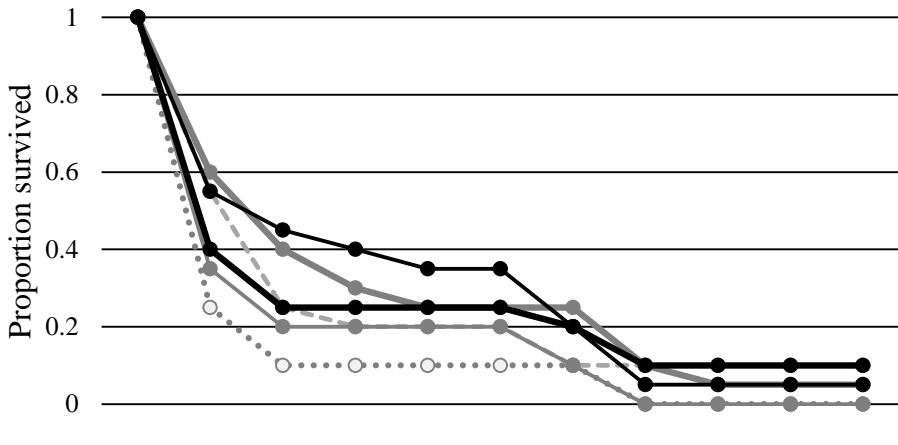


Fig. 2. Map of outplanting sites on Hawai'i Island. Seedlings were reciprocally outplanted at six elevations. Two outplanting sites (950 and 2050 m) differed from the collection sites because outplanting at the latter (managed by Hawai'i Volcanoes National Park) was not permitted.

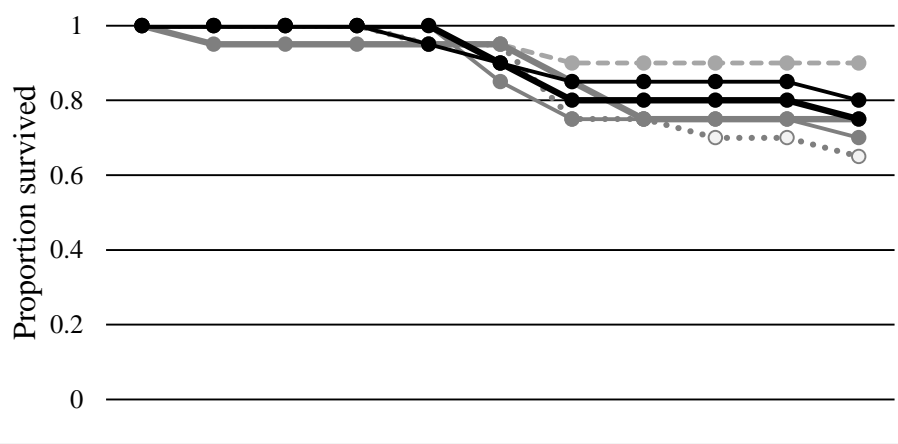
2300 m



2050 m



1575 m



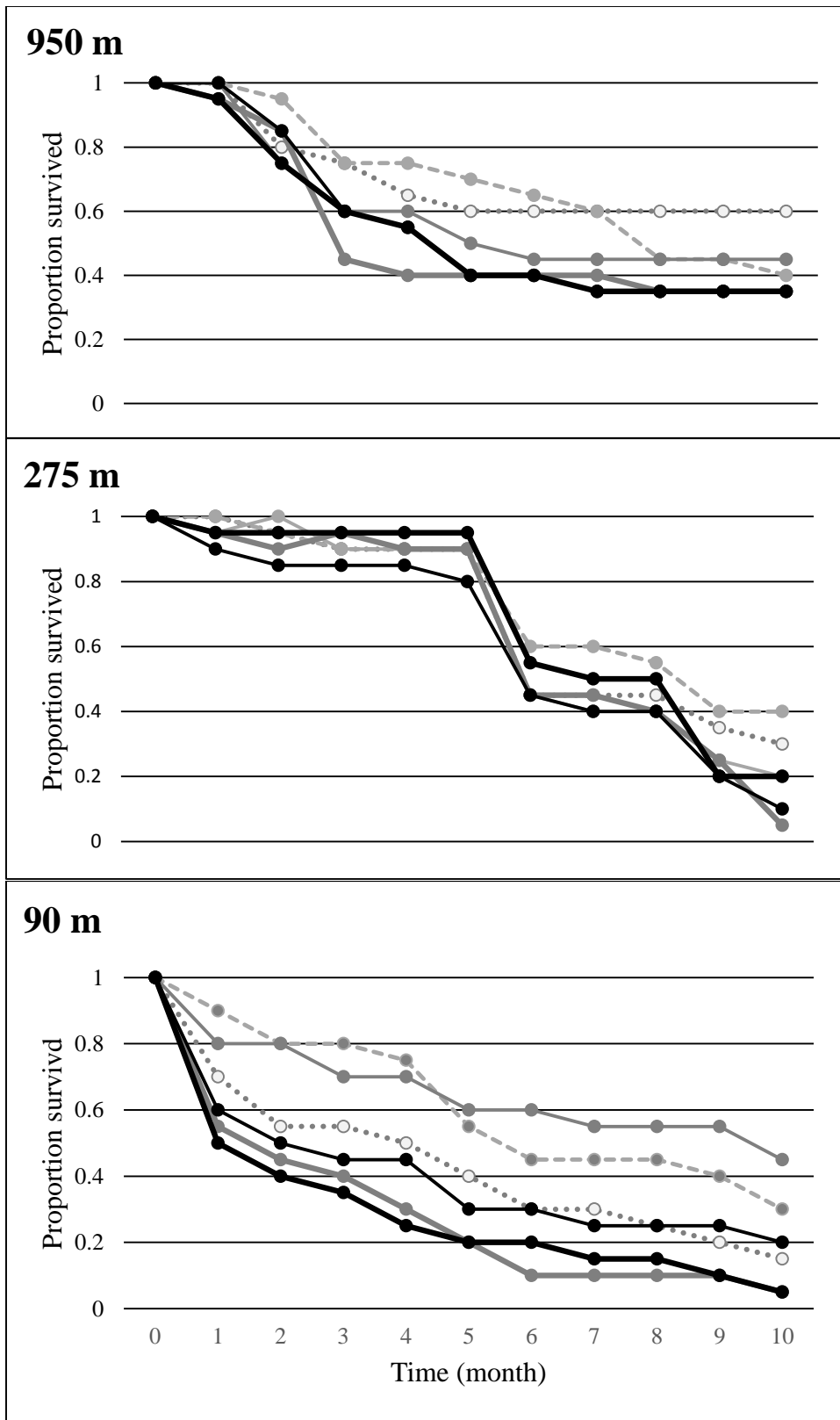


Fig 3. Mean 10-month survivorship of outplanted seedlings at six elevations on East side of Hawai'i Island from 2014-2015.

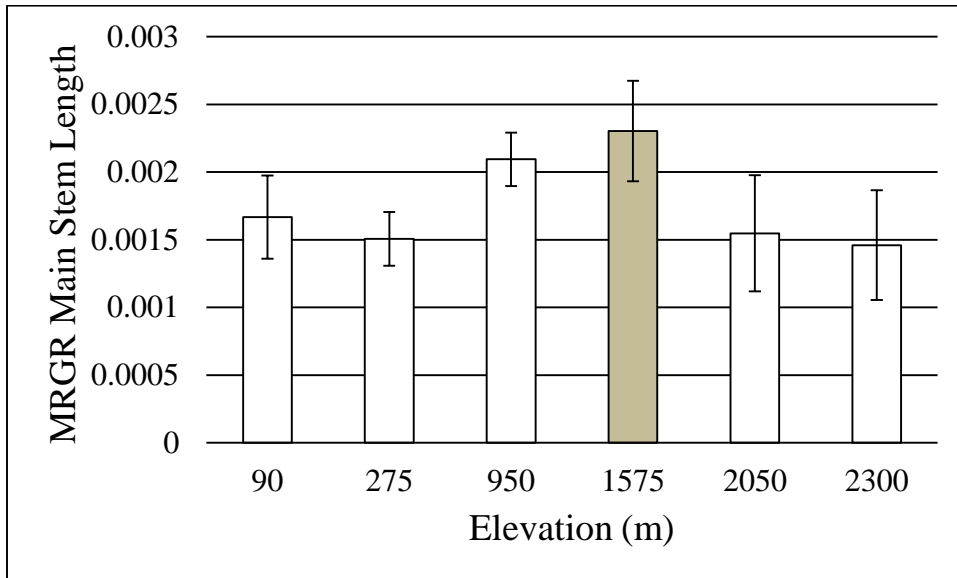


Fig. 4. Mean \pm SE of relative growth rate (MRGR) of main stem length of seedlings planted at 1575 m in elevation on Mauna Loa, Hawai'i after 12 months of reciprocal transplant experiment.

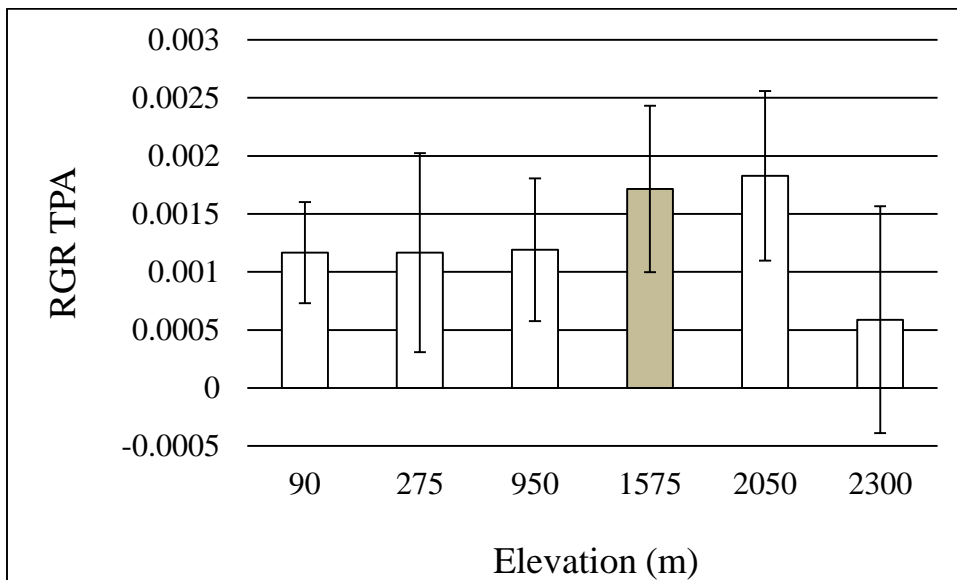


Fig. 5. Mean \pm SE of MRGR of TPA in seedlings planted at 1575 m in elevation on Mauna Loa, Hawai'i after 12-month of reciprocal transplant experiment.

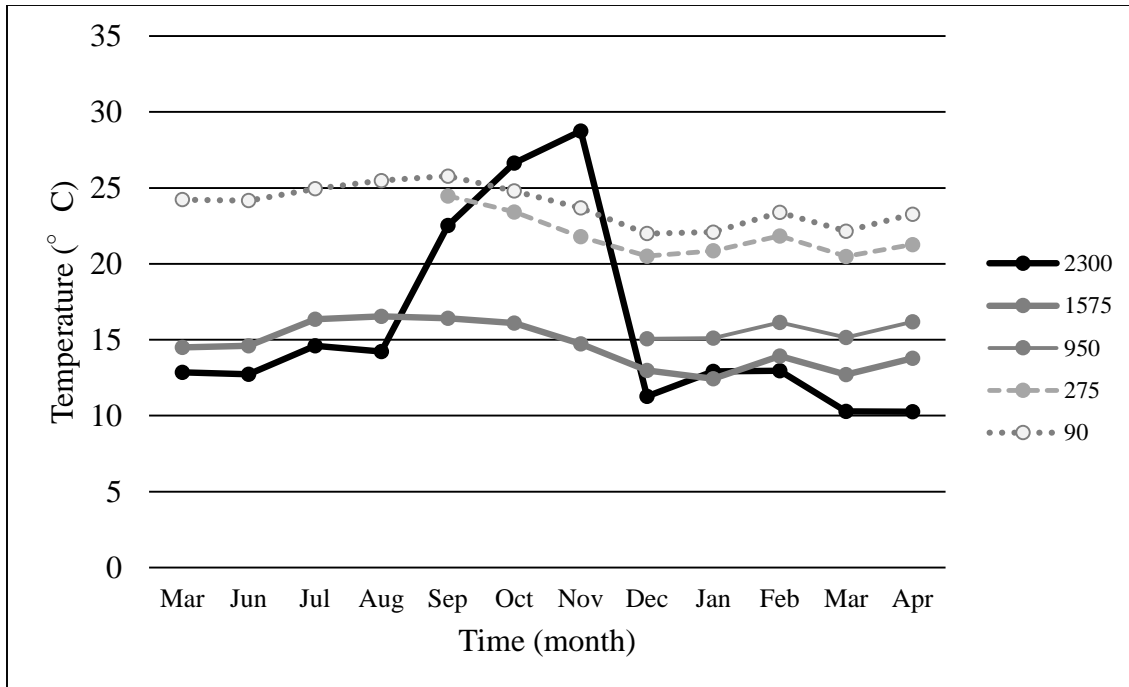


Fig. 6. Mean temperature per month recorded using HOBO Weather Stations placed at each outplanting site from March 2014 to April 2015.

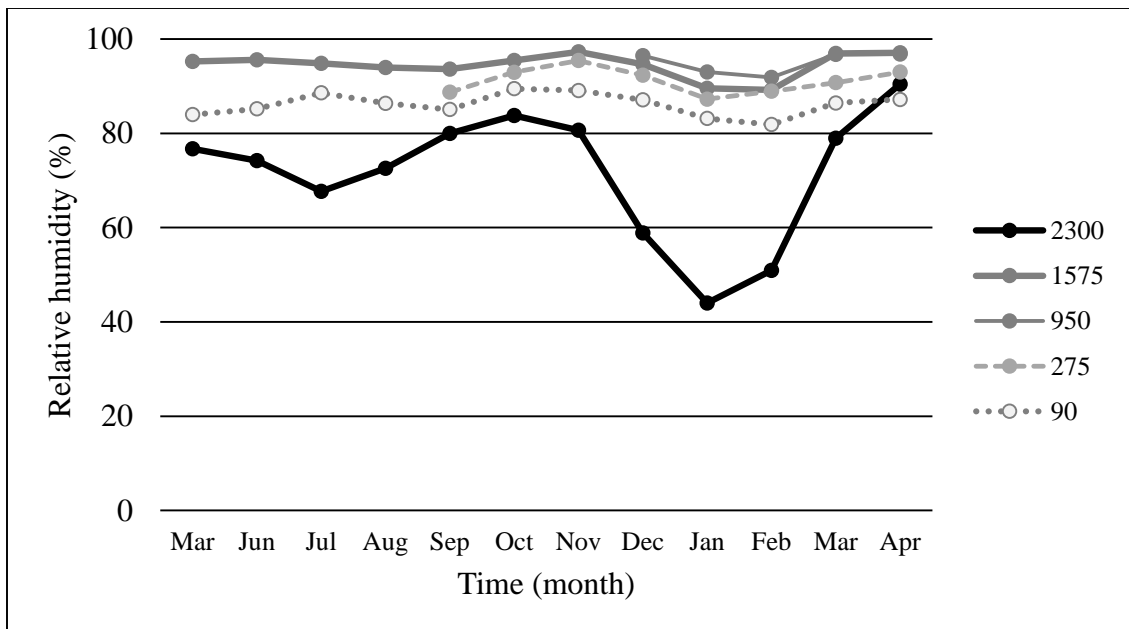


Fig. 7. Mean relative humidity per month recorded using HOBO Weather Stations placed at each outplanting site from March 2014 to April 2015.

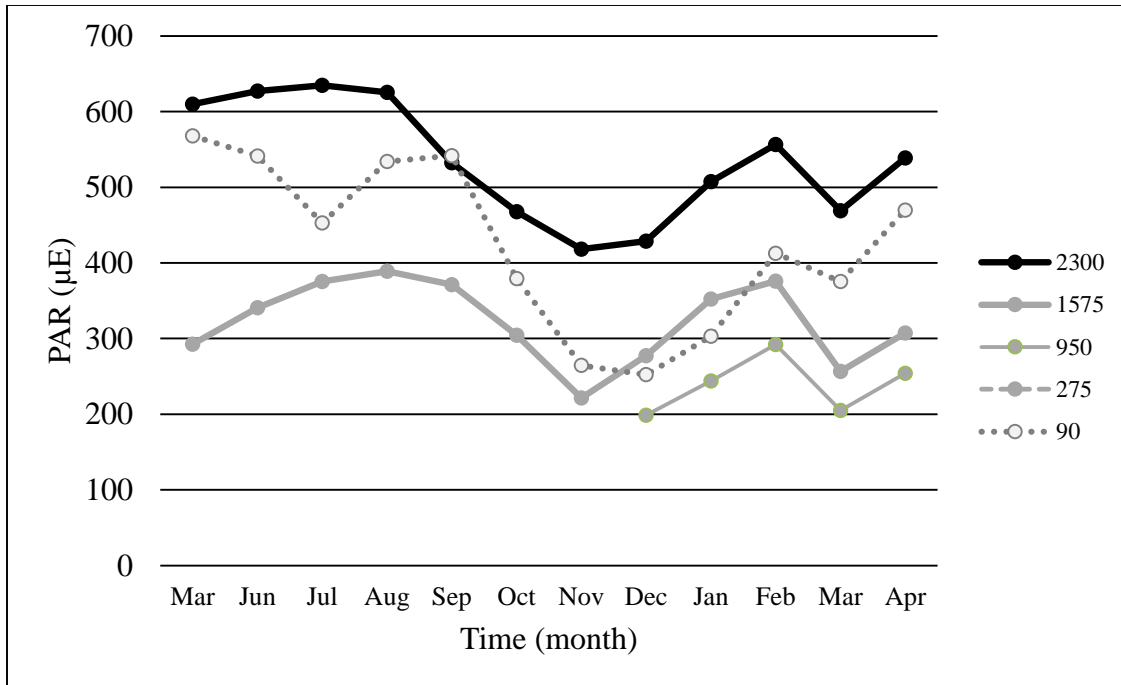


Fig. 8. Mean photosynthetically active radiation (PAR) per month recorded using HOBO Weather Stations placed at each outplanting site from March 2014 to April 2015.

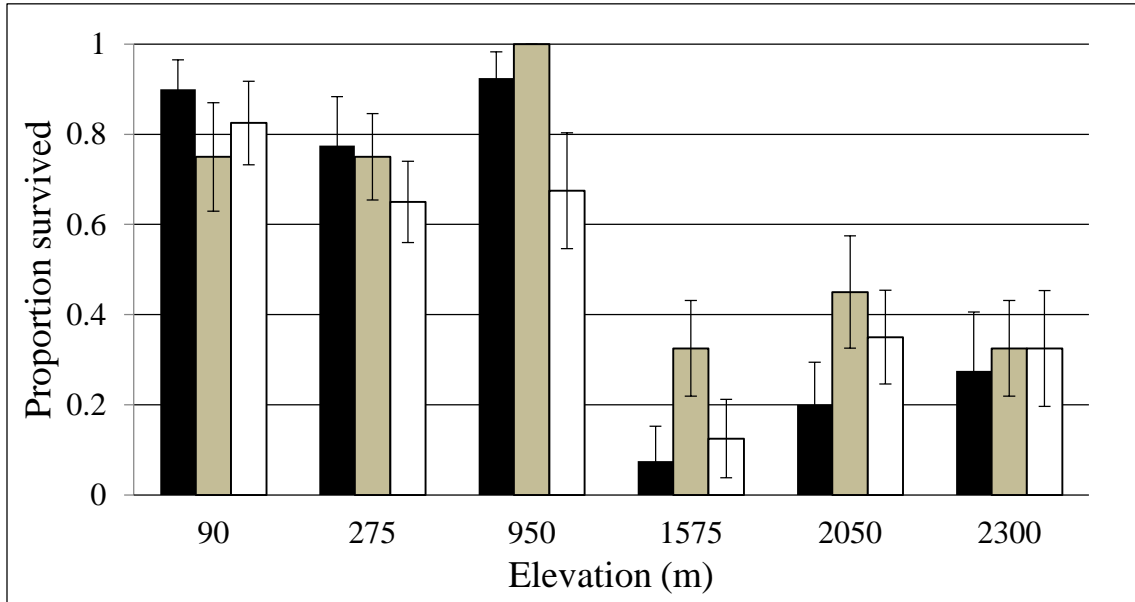


Fig. 9. Mean \pm SE proportion survivorship of seedlings grown from open-pollinated seeds from six elevations after the 12-month greenhouse experiment under three UV light levels. Note: black = survivorship under extra UV, gray = under ambient, and white = under no UV.

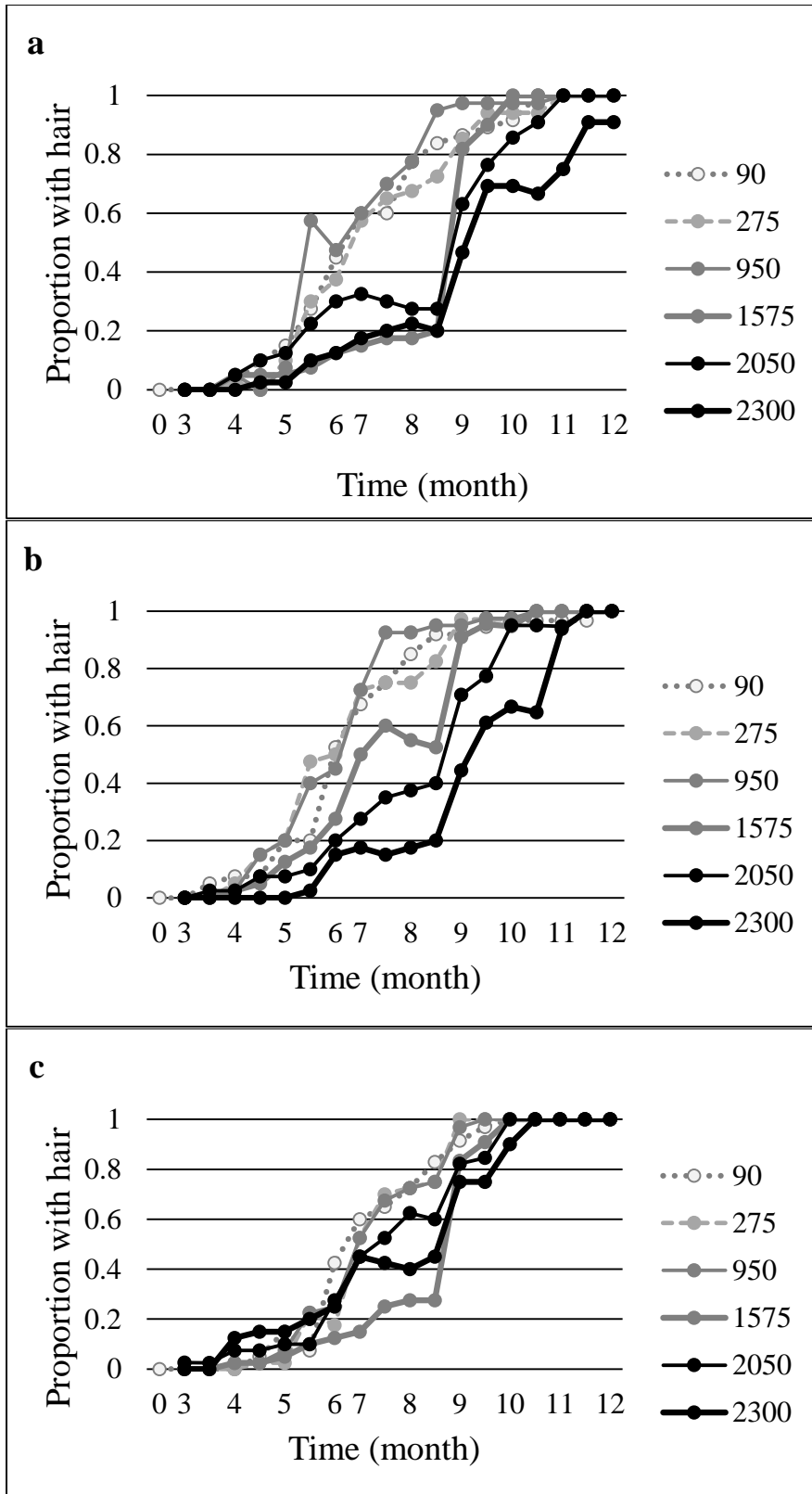


Fig. 10. Development of leaf pubescence over time on seedlings derived from open-pollinated seeds from six elevations and grown for 12 months in the greenhouse under three UV light levels: a) high UV, b) ambient UV, and c) no UV.

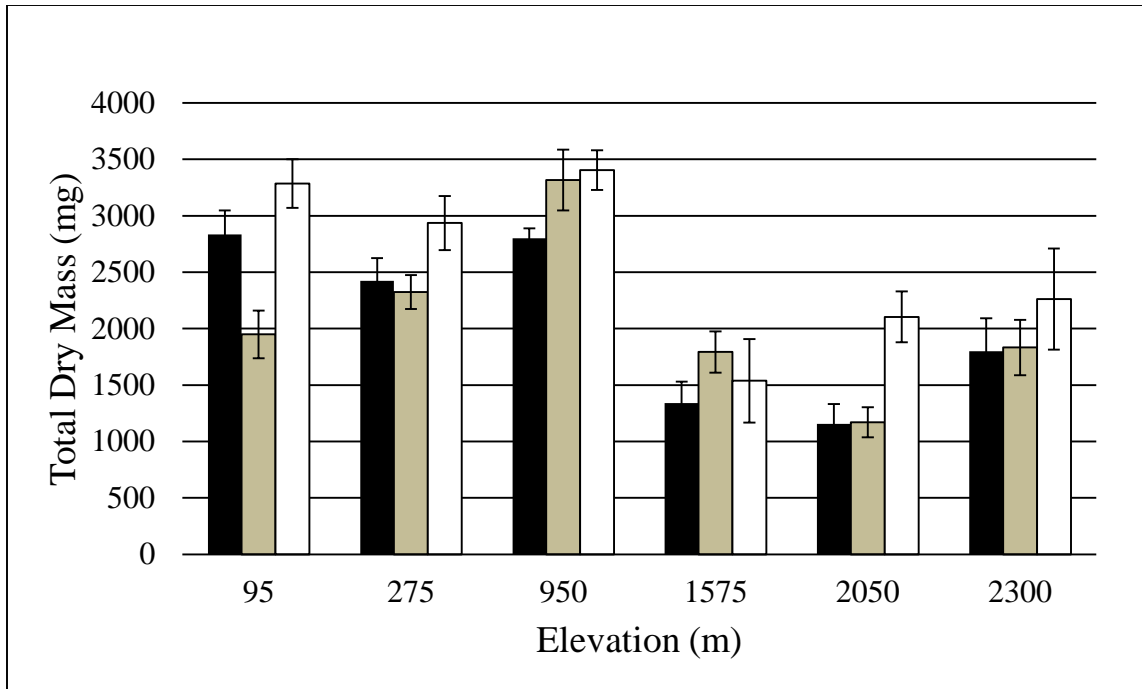


Fig. 11. Total dry mass of seedlings averaged among a population after the 12-month greenhouse experiment under three UV light levels. Note: black = under extra UV, gray = ambient UV, and white = no UV.

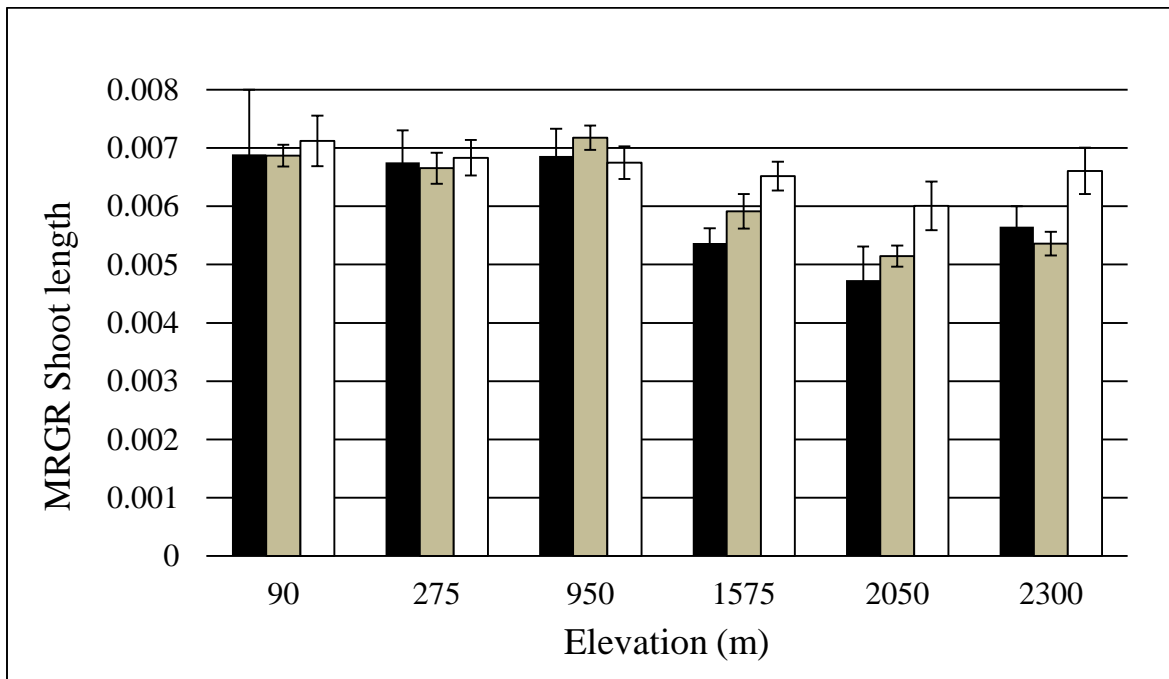


Fig. 12. Mean \pm SE MRGR of main stem length of seedlings from six elevations after the 12-month greenhouse experiment under three UV light levels. Note: black = under extra UV, gray = ambient UV, and white = no UV.

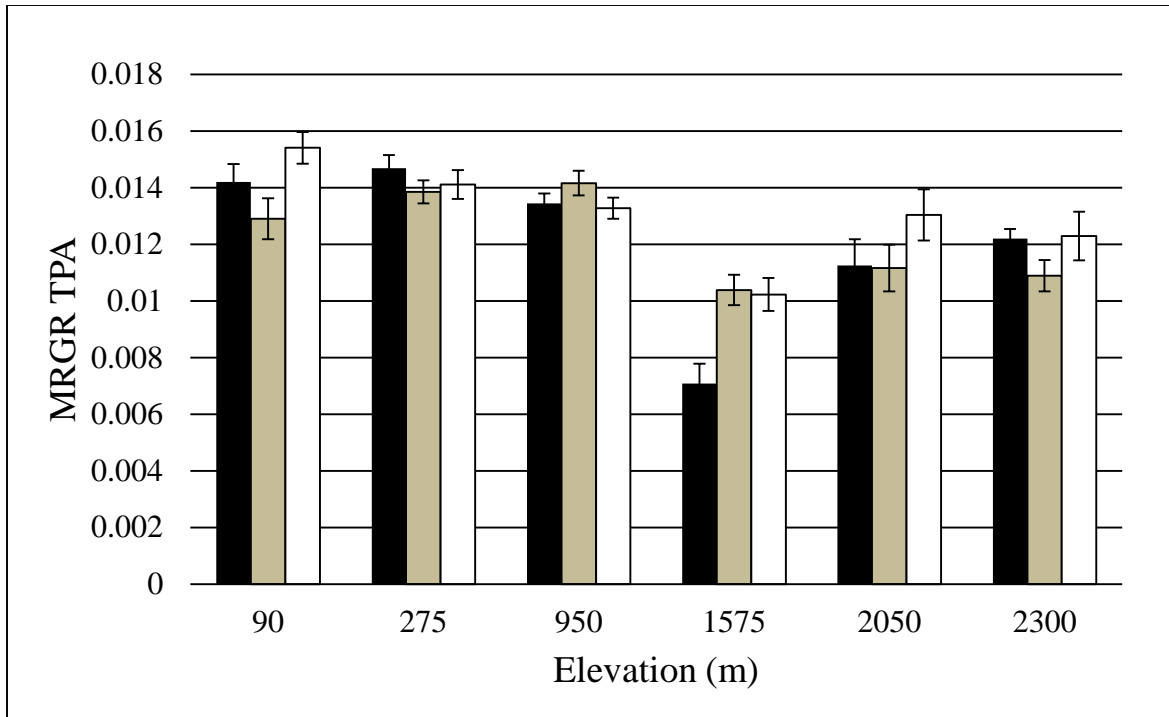


Fig. 13. Mean \pm SE MRGR of total photosynthetic area of seedlings from six elevations after the 12-month greenhouse experiment under three UV light levels. Note: black = under extra UV, gray = ambient UV, and white = no UV.

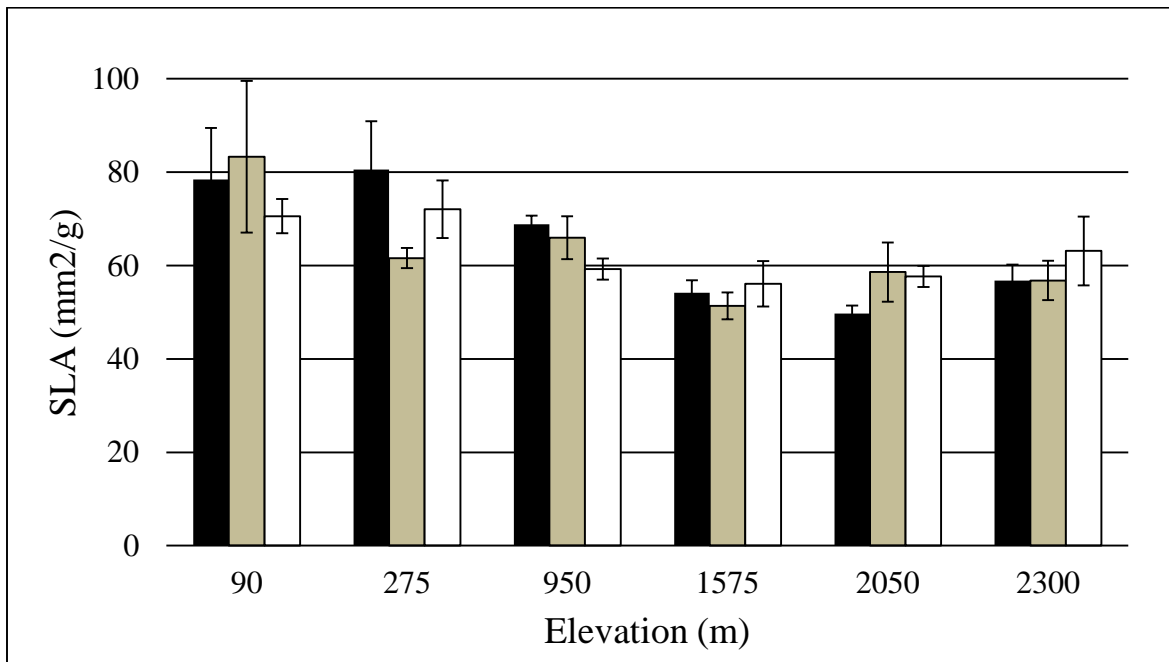


Fig. 14. Mean \pm SE SLA of seedlings from six elevations after the 12-month greenhouse experiment under three UV light levels. Note: black = under extra UV, gray = ambient UV, and white = no UV.

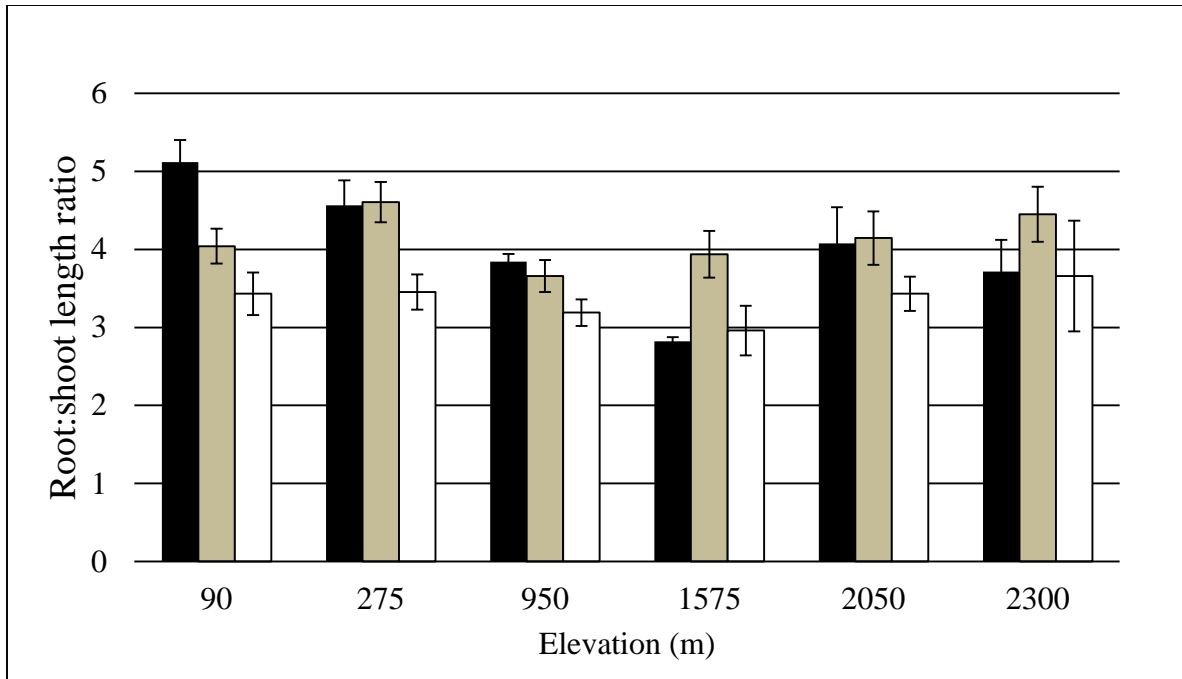


Fig. 15. Mean \pm SE root:shoot length ratio of seedlings from six elevations after the 12-month greenhouse experiment under three UV light levels. Note: black = under extra UV, gray = ambient UV, and white = no UV.

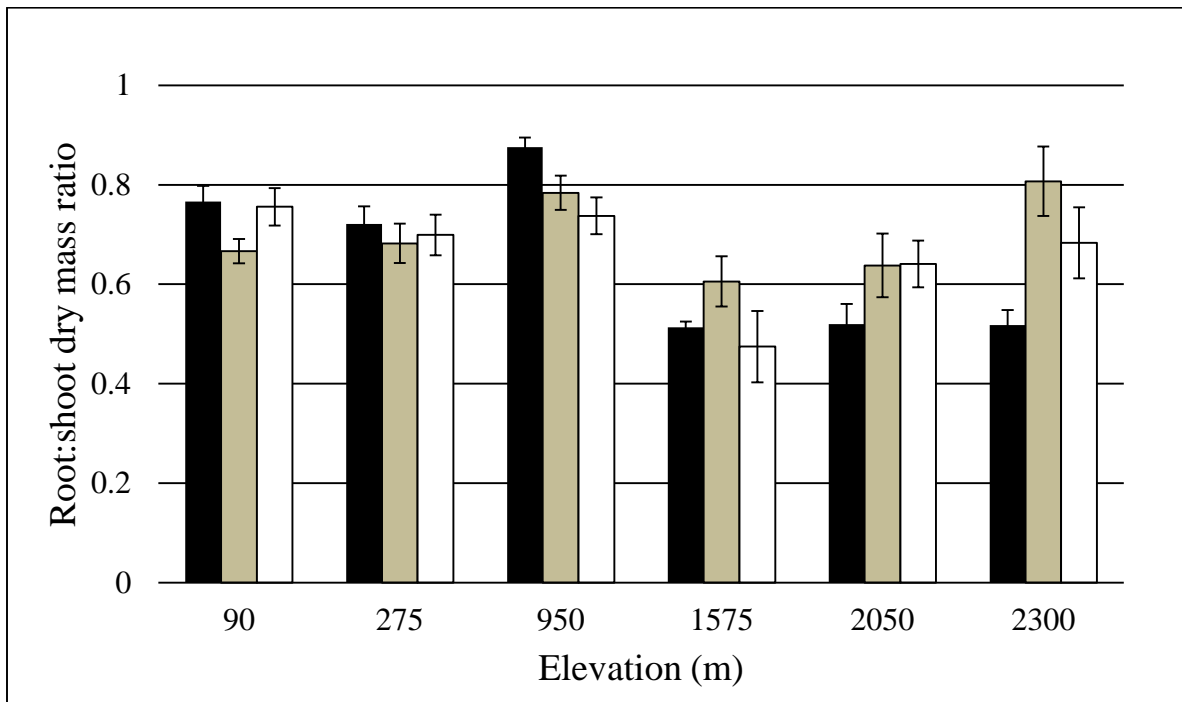


Fig. 16. Mean \pm SE root:shoot dry mass ratio of seedlings from six elevations after the 12-month greenhouse experiment under three UV light levels. Note: black = under extra UV, gray = ambient UV, and white = no UV.

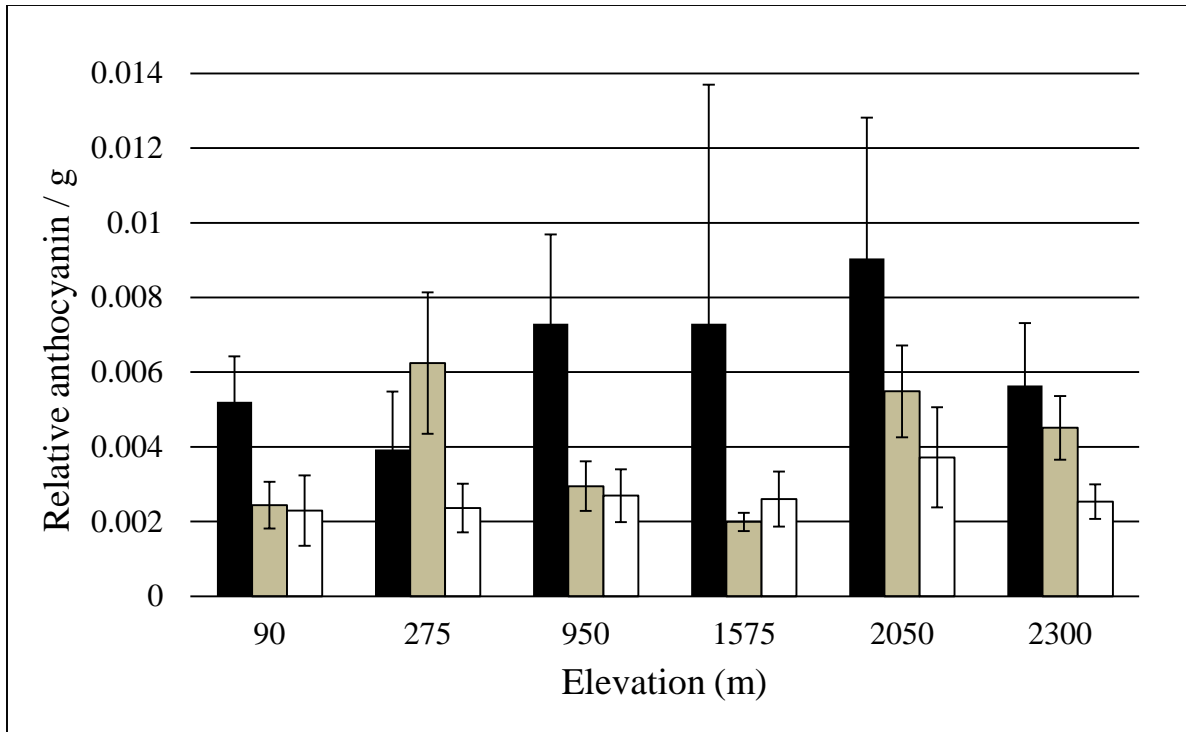


Fig. 17. Mean \pm SE relative anthocyanin content per gram of fresh leaf for seedlings from six elevations after the 12-month greenhouse experiment under three UV light levels. Note: black = under extra UV, gray = ambient UV, and white = no UV.

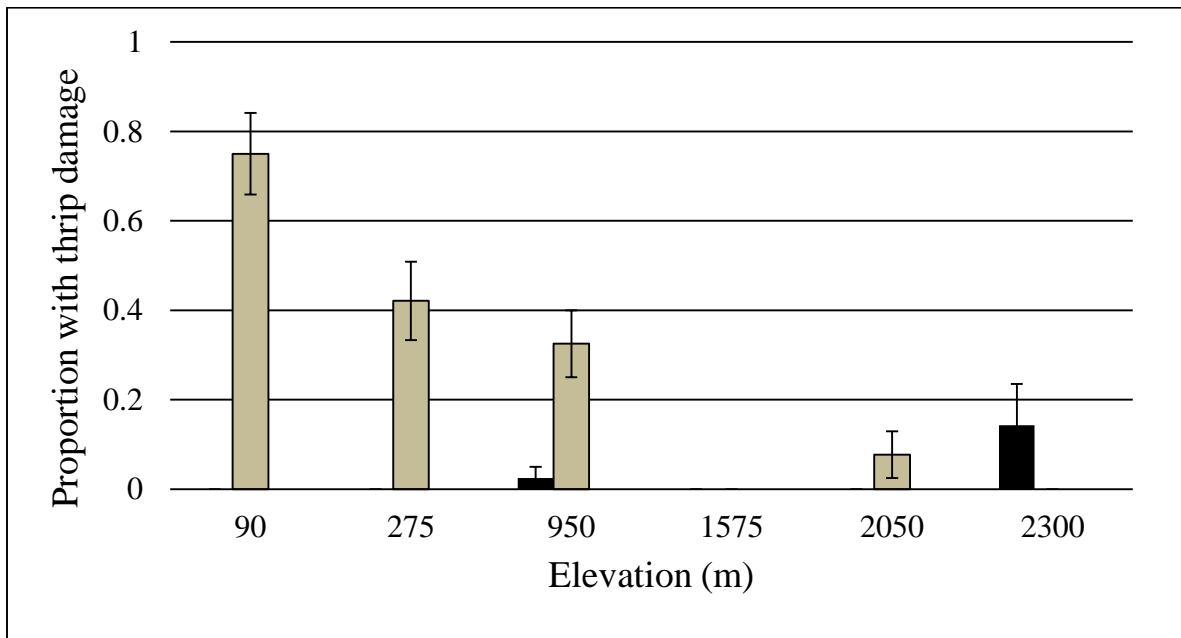


Fig. 18. Mean \pm SE proportion of seedlings showing thrips damage on leaves at end of the 12-month greenhouse study of UV light effects. Note: black = under extra UV, gray = ambient UV, and white = no UV.

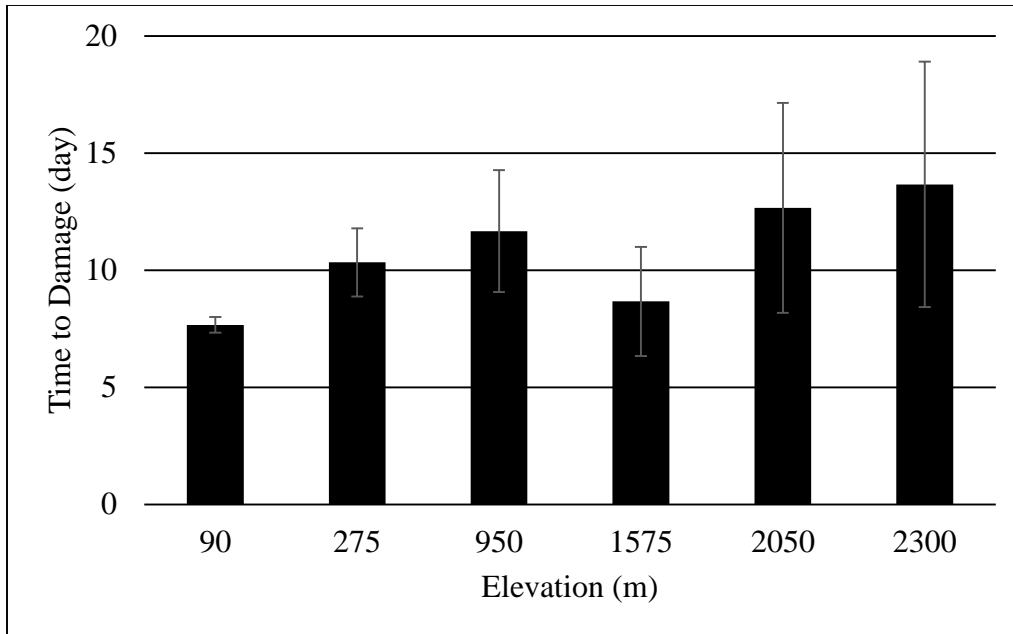


Fig. 19. Mean \pm SE time (days) to damage of seedlings from six elevations in response to UV radiation in the growth chamber.

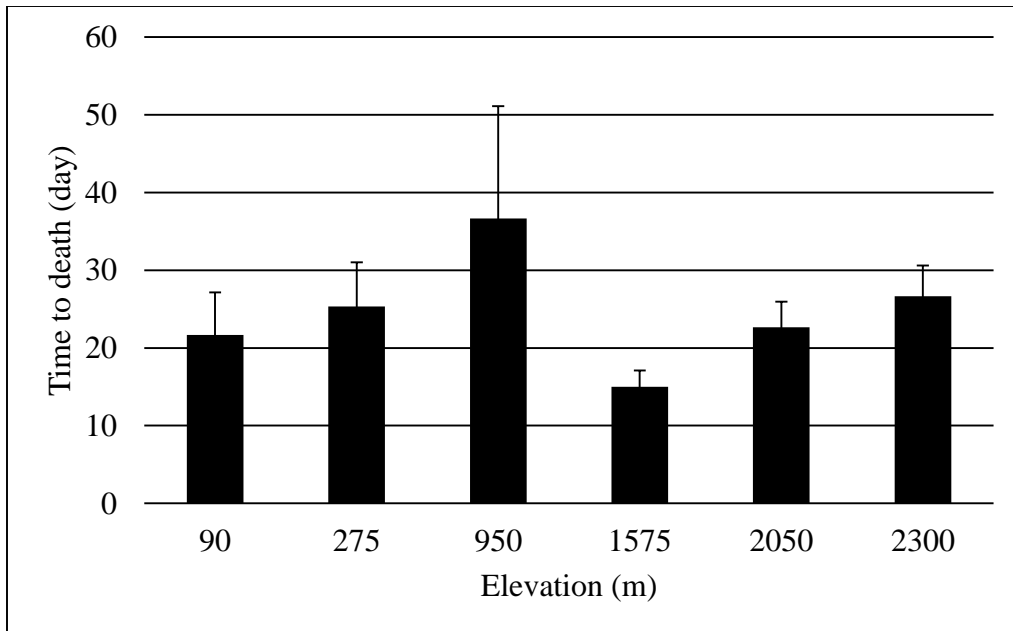


Fig. 20. Mean \pm SE time (days) to death of seedlings from six elevations in response to UV radiation in the growth chamber.

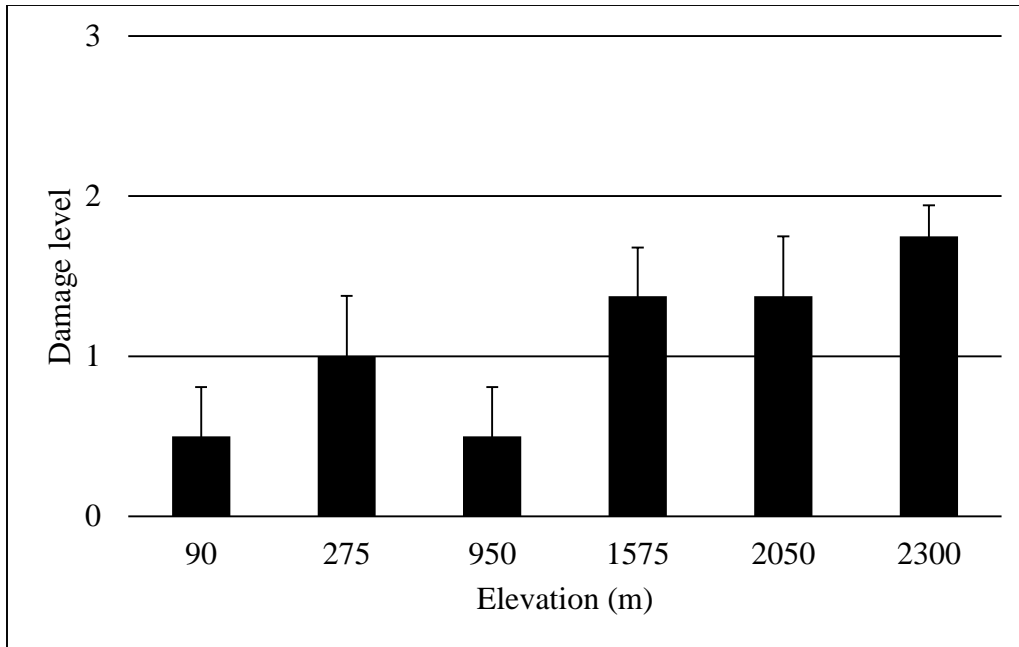


Fig. 21. Mean \pm SE damage level of seedlings from six elevations four days after exposure to freezing (-5 C). All control seedlings (not shown) survived the experiment. Damage levels are: 3 = alive, 2 = damaged, 1 = dying, and 0 = dead.

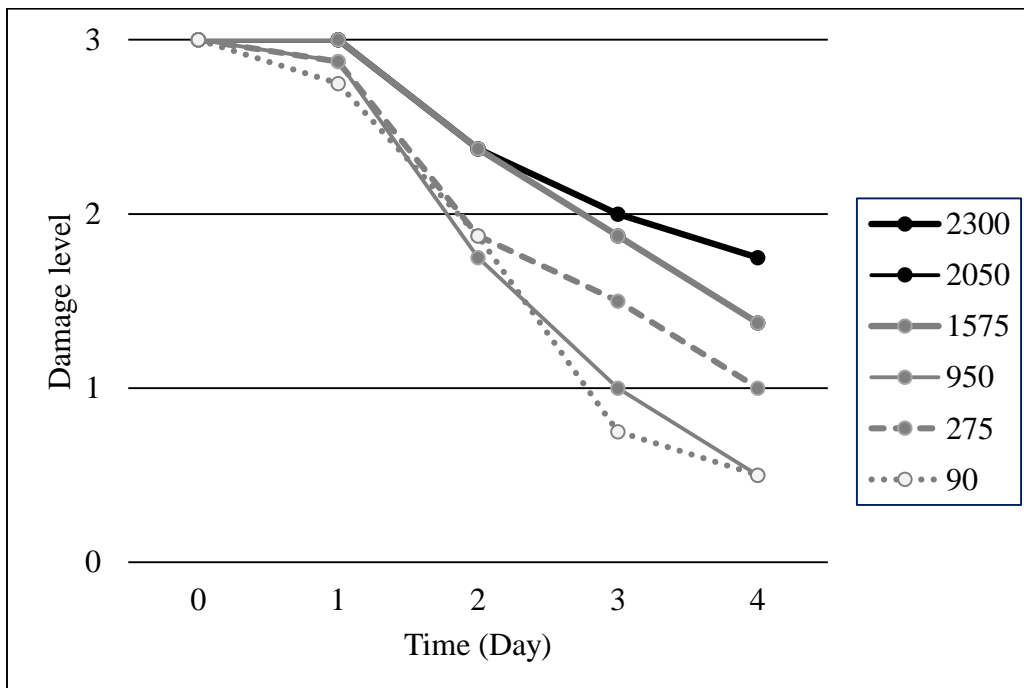


Fig. 22. Seedling response to freezing temperature (~ -5 °C) in the growth chamber over time shown as damage level. Damage levels were recorded as 3 = alive, 2 = damaged, 1 = dying, and 0 = dead for each seedling.

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